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Edwin.L.Mongan-1@usa.dupont.com

April 7, 2005

Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 2216

Attn: Chemical Right-to-Know Program

Re: Test Plan and Robust Data Summary for Fluorobenzene

Dear Administrator Leavitt,

E. I. du Pont de Nemours & Company, Inc. has completed the recommended testing for Fluorobenzene, and are pleased to submit a revised robust data summary.

With this submission we have completed the required data set and fulfilled our HPV commitment for this chemical.

Please feel free to contact me with any questions or concerns you may have with regards to this submission at Edwin.L.Mongan-1@usa.dupont.com or by phone at 302-773-0910.

Sincerely,

Edwin L. Mongan, III
Manager, Environmental Stewardship
DuPont Safety, Health & Environment

Cc: Charles Auer – U.S. EPA
Office of Pollution Prevention & Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

(April 7, 2005)

OVERALL SUMMARY FOR FLUOROBENZENE

Summary

Fluorobenzene is a liquid with a water solubility of approximately 1540 mg/L. Fluorobenzene has a freezing point of -40ºC, boiling point of 84.73ºC at 760 mm Hg, density of 1.024 g/mL, and a vapor pressure of 100 mm Hg at 30.4ºC.

A review of estimated physical-chemical properties and environmental-fate characteristics indicates that fluorobenzene may be persistent in air with an estimated half-life due to hydroxyl radical oxidation of 23.3 days. Based on the BIOWIN ultimate survey model estimate of weeks-to-months, fluorobenzene may be moderately persistent in terrestrial compartments, and is not expected to readily biodegrade (Table 1). Fluorobenzene is not highly bioaccumulative with an estimated BCF of 11.17 (Table 1). When modeled using a Level III fugacity model under a standard scenario of equal emissions to air, water, and soil, fluorobenzene is expected to partition primarily into air and water compartments (Table 1). The Hydrowin model (v. 1.67, Syracuse Research Corporation) could not estimate a hydrolysis rate for fluorobenzene in regard to stability in water. However, halogenated aromatics/PCBs are generally resistant to hydrolysis (Harris, 1990), and thus fluorobenzene would be likely to be stable to hydrolysis in water. A hydrolysis test using OECD Guideline 111 was conducted and confirmed that fluorobenzene is hydrolytically stable in water.

Table 1 : Environmental Fate

Bioconcentration*	BCF = 11.17
Biodegradation*	Does not readily biodegrade
Fugacity*	Level III Partition Estimate Air 40.9 % Water 44 % Soil 14.8 % Sediment 0.245 %
* Modeled data	

In aquatic organisms, fluorobenzene has low toxicity to fish with a 96-hour LC_{50} in fathead minnows of 210 mg/L. Fluorobenzene was moderately toxic to *Daphnia* in a 24-hour study which produced an EC_{50} of 7.37 mg/L. Modeling of physical-chemical parameters (i.e., log Kow) and aquatic toxicity was conducted to help provide insight into the behavior in the environment and the aquatic toxicity of fluorobenzene (See Table 2). Syracuse Research Corporation models for estimating physical-chemical properties were used to estimate $\log_{10}$ Kow (Meylan and Howard, 1995) for subsequent use in the ECOSAR program. ECOSAR (Meylan and Howard, 1999) was used to estimate aquatic toxicity data for green algae, daphnids (planktonic freshwater crustaceans), and fish. ECOSAR predictions are based on actual toxicity

test data for classes of compounds with similar modes of action. The predicted $\log_{10}$ Kow value was used as input for the ECOSAR model (see Table 2 for values). The ECOSAR predictions indicate that fluorobenzene is of low to medium concern relative to acute toxicity to algae, invertebrates, and fish.

Additional aquatic toxicity data are presented for another mono-substituted halobenzene (chlorobenzene) as well as several di-substituted halobenzenes (chloro-fluorobenzenes). The acute fish, daphnid, and algae data for chlorobenzene support the data presented for fluorobenzene. Although the fluorobenzene and chlorobenzene acute test data for daphnids are from 24-hour tests, the 48-hour chloro-fluorobenzene test data (based on measured test concentrations) support the data for the monosubstituted halobenzenes. The dichlorobenzenes have been reported to be more toxic than monochlorobenzene (Galassi and Vigli, 1981) although position of the chloro substituted groups on the benzene ring appears to have no affect on aquatic toxicity (US EPA, 1980). This pattern also appears to hold true for the chloro-fluorobenzenes since they appear to be more toxic than either chlorobenzene or fluorobenzene and the position of the fluoro groups on the benzene ring has no effect on aquatic toxicity. ECOSAR appears to underestimate the toxicity of these compounds to daphnids, but the experimental data are adequate for acute hazard assessment. The existing data (experimental and estimated) are also adequate for assessing acute hazard to fish and algae, therefore no additional testing is necessary.

Table 2: Aquatic Toxicity Values

	Fluorobenzene	Chlorobenzene	1-Chloro-2-fluorobenzene	1-Chloro-3-fluorobenzene	1-Chloro-4-fluorobenzene
Log Kow	2.19	2.64	2.84	2.84	2.84
Toxicity to Fish (LC_{50} value)	96-hour: 210 mg/L (N) 48-hour: 430.5 mg/L (N) 96-hour: 47.2 mg/L (E)	96-hour: 10.4 mg/L (N) 96-hour: 20.9 mg/L (E)	No test data. 96-hour: 15.7 mg/L (E)	No test data. 96-hour: 15.7 mg/L (E)	No test data. 96-hour: 15.7 mg/L (E)
Toxicity to Invertebrates (EC_{50} value)	24-hour: 7.37 mg/L (M) 48-hour: 51.3 mg/L (E)	24-hour: 4.3 mg/L (N) 48-hour: 23.4 mg/L (E)	48-hour: 2.28 mg/L (M) 48-hour: 17.8 mg/L (E)	48-hour: 3.64 mg/L (M) 48-hour: 17.8 mg/L (E)	48-hour: 1.70 mg/L (M) 48-hour: 17.8 mg/L (E)
Toxicity to Algae (EC_{50} value)	No test data. 96-hour: 32.4 mg/L (E)	96-hour: 12.5 mg/L (N) 96-hour: 15.2 mg/L (E)	No test data. 96-hour: 11.7 mg/L (E)	No test data. 96-hour: 11.7 mg/L (E)	No test data. 96-hour: 11.7 mg/L (E)
E = estimated value, N = value based on nominal test concentrations. M = value based on measured test concentrations.					

Fluorobenzene has very low acute oral toxicity with an acute lethal dose (ALD) > 11,000 mg/kg in rats. No clinical signs of toxicity were observed in the non-lethal doses; however, slight to severe weight loss was noted. Fluorobenzene also had very low acute inhalation toxicity with a 4-hour acute lethal concentration (ALC) of 6200 ppm in rats. When applied to the skin of rabbits, fluorobenzene produced no to mild erythema and no to severe edema and was considered a mild skin irritant. Fluorobenzene did not induce dermal sensitization in guinea pigs. Fluorobenzene was moderately irritating to the rabbit eye in one study, and severely irritating in two other studies containing limited information. More severe effects were observed in eyes that were washed than those that remained unwashed.

In the following sections, subchronic studies on fluorobenzene and chlorobenzene are presented to support the fluorobenzene toxicity database. Developmental, reproductive, and chronic studies are available only for chlorobenzene. Chlorobenzene should be an acceptable structurally similar analog to support the HPV database for fluorobenzene for the following reasons. The metabolism of monohalobenzenes in mammals has been well studied. Qualitatively, monohalobenzenes, including chloro-, bromo-, iodo-, and fluorobenzene are all metabolized by common metabolic pathways in mammals. All halobenzenes are initially oxidized to the corresponding halophenol, either directly or via an intermediate epoxide. The epoxide intermediates may be hydrolyzed to dihydrodiols which are in turn oxidized to catechols, or may undergo conjugation with glutathione, resulting in the excretion of the corresponding mercapturic acid in the urine. A more detailed discussion of the metabolism of halobenzenes is presented at the end of the toxicity section. Overall, the pathways for metabolism of halobenzenes, including chlorobenzene and fluorobenzene are essentially identical. Thus chlorobenzene toxicity studies should be acceptable to support the fluorobenzene HPV database.

In a 28-day repeated dose study, groups of rats were exposed nose-only, 6 hours a day to 0.4 mg/L (94 ppm), 1.5 mg/L (381 ppm), or 6.0 mg/L (1585 ppm) of fluorobenzene. Slight changes in physical condition were seen for rats exposed to 1.50 or 6.24 mg/L. Other effects of treatment were confined to adaptive liver changes and unique male rat hydrocarbon nephropathy. Although the adaptive liver changes extended into the low dose group (0.37 mg/L), neither of these conditions were considered to be indicative of toxicologically important adverse effects of treatment and, consequently, the NOAEL was considered to be 0.37 mg/L (94 ppm). Furthermore, the slight changes observed in physical condition were not indicative of serious damage to the health of the animals. There was, however, evidence of a treatment-related increase in fluoride concentration in bones and teeth of animals from all exposure groups.

In a 13-week subchronic study, groups of ten male and ten female Fisher 344 rats or B6C3F1 mice were dosed with 0, 60, 125, 250, 500 or 750 mg/kg/day chlorobenzene. The NOEL was 125 mg/kg/day. Effects observed at higher doses in rats included reduced body weight gain, reduced survival, clinical chemistry changes, organ weight changes, and pathological changes in liver, kidney, thymus, bone marrow, and spleen. In a 13-week inhalation study, 15 Charles River rats/gender/group and 4 beagle dogs/gender/group were exposed to 0, 0.75, 1.5, or 2.0 mg/L chlorobenzene 6 hours/day, 5 days/week. Effects observed in rats at 0.75 mg/L or higher included hypoactivity, reduced body weight, and organ weight changes. There were no gross or

histologic changes in rats attributable to the test substance. Effects observed in dogs at 1.5 mg/L and higher included hypoactivity, reduced body weight, increased liver weight, yellow discoloration of aorta, hardened livers, and, in the high dose group, histologic changes of the liver, bone marrow, kidney, and testes. Seven dogs in the mid- and high group were sacrificed *in extremis*.

In a chronic study, groups of 50 Fischer 344 rats or B6C3F1 mice/gender/group were dosed with 0, 30, or 60 mg/kg/day (male mice) or 0, 60, or 120 mg/kg/day (female mice, male and female rats). There were no toxic effects in male or female mice. Although decreased survival was observed in male mice it was not correlated with toxicity or body weight effects. Therefore there did not appear to be a causal relationship between chlorobenzene exposure and reduced survival. Tumors common in aged mice occurred with similar frequency in all groups and were not considered related to chlorobenzene exposure. In rats, survival was decreased in the high-dose group. The only tumor type to occur with increased frequency was neoplastic nodules of the liver. In addition, a papilloma of the urinary bladder (one each in the 60 and 120 mg/kg/day male rats) and a tubular cell adenocarcinoma in one female 120 mg/kg/day female rat were observed. These were rare tumor types that did not occur in vehicle or untreated control rats.

No data on potential developmental toxicity of fluorobenzene were available. However, several developmental toxicity studies have been conducted using the close structural analogue chlorobenzene. These compounds have similar log Kow values (2.19 and 2.64 for fluoro- and chlorobenzene, respectively), suggesting that maternal/fetal partitioning for these compounds is likely to be similar. Fluoro- and chlorobenzene are also metabolized by similar pathways, with *para*- and *ortho*-phenols as the major products (Koerts et al., 1997; Rietjens et al., 1993). Phenolic metabolites of both compounds are subsequently conjugated with glucuronic acid and excreted. These similarities suggest that chlorobenzene should serve as a suitable model for fluorobenzene with regard to prediction of developmental toxicity. This conclusion is supported by *in silico* analysis of fluoro- and chlorobenzene using TOPKAT (Health Designs Inc, Rochester, NY) and MultiCASE (MULTICASE Inc., Cleveland OH). TOPKAT predicted both compounds to be negative for developmental toxicity in mammals. Similarly, MultiCASE predictions using modules for rabbit, rat, and mouse teratogenicity were negative for both halobenzenes.

The developmental toxicity studies for chlorobenzene indicated that chlorobenzene was not a unique developmental toxin in either rats or rabbits. Pregnant female Fisher-344 rats were exposed to 0, 75, 210, or 590 ppm chlorobenzene in air for 6 hours/day on days 6-15 of gestation. The maternal NOAEL was 210 ppm. Maternal toxicity occurred at 590 ppm as evidenced by decreased body weight gain on gestation days 6-8 and increased absolute and relative liver weights at study termination on gestation day 21. The fetal NOAEL was also 210 ppm based on an increase in skeletal variations (delayed ossification of vertebrae centra and bilobed thoracic centra) at 590 ppm. These variations were indicative of a slight delay in skeletal development among the fetuses (mild fetotoxicity) at a maternally toxic dose. The incidence of malformations (collectively or individually) was not increased in any of the exposed groups.

In addition, two inhalation developmental studies on chlorobenzene were conducted in rabbits. Pregnant female New Zealand white rabbits were exposed to 0, 75, 210, or 590 ppm

chlorobenzene in air (first study) or 0, 10, 30, 75 or 590 ppm chlorobenzene in air (second study) for 6 hours/day on days 6-18 of gestation. The maternal NOAEL was 75 ppm based on significantly increased absolute and relative liver weight at 210 and 590 ppm at study termination on gestation day 29. In the first study, a few chlorobenzene-exposed fetuses exhibited visceral malformations which were not observed among concurrent controls. However, there was no dose-related increase in malformations and there was no increase in malformations in chlorobenzene-exposed groups in the subsequent study. In the second study there was a significant increase in litters with resorptions at 590 ppm, although this effect was not observed at any concentration on the first study, and was within the range of historical controls. Therefore the conclusion was that chlorobenzene did not have an embryotoxic effect on rabbits.

While no formal reproductive toxicity studies have been conducted on fluorobenzene, no reproductive effects were observed in testes or ovaries in a 28-day inhalation study in rats. A 2-generation reproduction study with chlorobenzene was conducted by the inhalation route. Thirty male and female Sprague-Dawley rats per group were exposed to 0, 50, 150, or 450 ppm (0, 234, 702, or 2105 mg/m^3) 6 hours/day, 7 days/week. There were no substance-related effects on mortality, body weights, or food consumption. Mating, fertility, and other reproductive parameters and pup survival appeared unaffected by treatment. Liver weights were increased at 150 ppm and above in both generations and also at 50 ppm in the second generation male rats. The authors stated that the biological significance of the increased liver weights for the 150 and 450 ppm females and 50 ppm males was unclear. Liver and renal changes at 150 ppm and above and testicular effects at 450 ppm were observed in both generations. The authors state that the relationship of these testicular changes to chlorobenzene exposure was unclear because there did not appear to be any increase in intensity and/or incidence of testicular lesions among F1 adults that had longer exposure. The no adverse effect level was 50 ppm for F0 and F1 rats and >450 ppm for F2 offspring.

Fluorobenzene was equivocal in the Ames test. Fluorobenzene was tested in a preincubation assay in *Salmonella* strains TA100, TA1535, and TA98, without metabolic activation and with rat and hamster liver activation; a positive result with hamster liver activation was observed in strains TA100 and TA1535. A second study was conducted with a wider range of doses where the chemical was tested up to the highest dose permitted by toxicity. Based on limited data, fluorobenzene was not clastogenic in a mouse bone marrow micronucleus test.

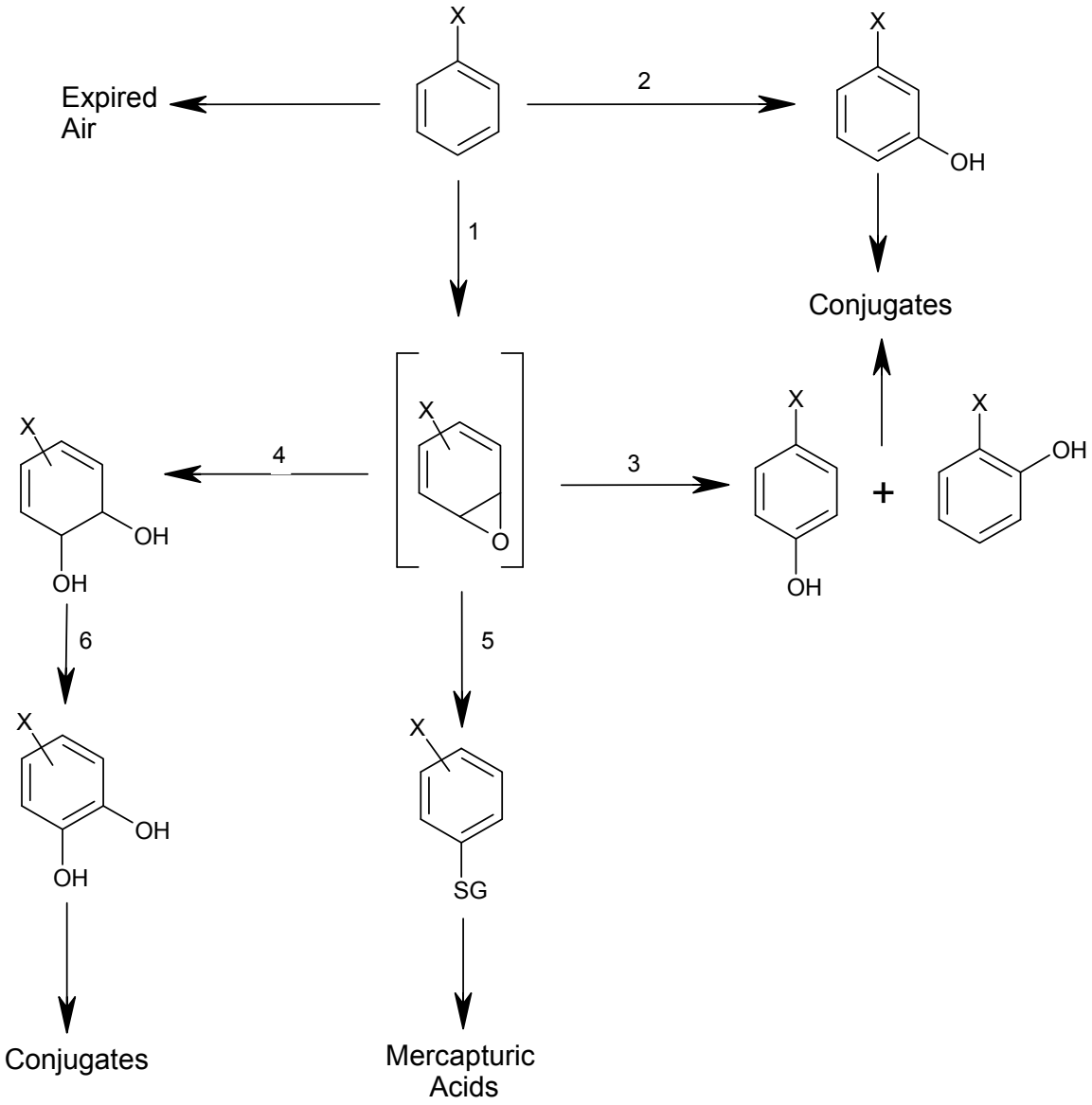
The following metabolism data is presented to support the use of monochlorobenzene as an acceptable surrogate compound to support the fluorobenzene database. The metabolism of monohalobenzenes has been the subject of extensive investigation for over 50 years (Yoshida and Hara, 1985; Krewet et al., 1989; Billings, 1985; Burka et al., 1983; Mills and Wood, 1953; Spencer and Williams, 1950; Kerger et al., 1988; Gut et al., 1996; Ogata et al., 1991; Ogata and Shimada, 1983; Koerts et al., 1997; Koerts et al., 1988; Rietjens et al., 1993). Qualitatively, monohalobenzenes, including chloro-, bromo-, iodo-, and fluorobenzene are all metabolized by common metabolic pathways (Figure 1). The initial step in halobenzene metabolism is hydroxylation at the C2, C3 or C4 position to form the corresponding 2-, 3- or 4-halophenol. Oxidation of halobenzenes to halophenols is though to occur via two mechanisms. Hydroxylation at C3 is thought to occur largely, if not entirely, by direct oxidation of the C3

position (pathway 2). Hydroxylation and C2 and C4 can occur through a direct oxidation of the appropriate carbon, but may also occur via formation of 2,3- and 3,4-epoxides, respectively (pathway 1). The epoxides undergo rearrangement through resonance stabilized cationic intermediates to form the corresponding 2- and 4-halophenols (pathway 3). The latter pathway accounts for the so called NIH shift seen with polyhalogenated benzenes (Koerts et al., 1998). For all halobenzenes, the major site for hydroxylation is the C4 position. The regioselectivity of oxidation of other carbon centers is driven by the Van der Waals radius of the halogen substituent. For larger halogens such as bromine and iodine, hydroxylation at C3 is favored over C2, while for smaller halogens such as fluorine the opposite is true. This is consistent with the findings of Burka et al., 1983 who demonstrated an inverse relationship between the size of the halogen substituent and the extent of hydroxylation at C2 for a series of monohalobenzenes. The involvement of epoxide intermediates is further supported by the identification of dihydrodiols (pathway 4) as urinary metabolites of chloro- and bromobenzene in rats, and *in vitro* following incubation of rat hepatocytes with halobenzenes (Billings, 1985; Zampaglione et al., 1973). The dihydrodiols thus formed can be oxidized by dihydrodiol dehydrogenase to form the corresponding catechols (pathway 5). Catechol formation is a relatively minor metabolic route in rats treated with chlorobenzene and fluorobenzene. However, 4-chlorocatechol is the major urinary metabolite of chlorobenzene in humans, suggesting that 4-fluorocatechol is likely to be a major human metabolite of fluorobenzene. These data are consistent with the relative expression levels of microsomal epoxide hydrolase in rats and humans. A final pathway for metabolism of halobenzene epoxides is conjugation of the epoxide moiety with glutathione (pathway 6). This reaction is accompanied by loss of water to form the S-halophenyl glutathione conjugate, which is excreted as the corresponding mercapturic acid in the urine.

In rats, approximately 20% of the administered dose of chlorobenzene is recovered as urinary metabolites within 24 hrs, with up to75% of the dose exhaled in the expired air (Krewet et al., 1989). Of the urinary metabolites, approximately 3% of the administered dose is recovered as phenolic metabolites, 11% as catechols and about 5% as mercapturic acids. The balance of urinary metabolites is composed of dihydrodiols and trace quantities of dehalogenated metabolites. Fluorobenzene metabolism in rats has been investigated using ^{19}F NMR, with emphasis on hydroxylated metabolites (Koerts et al., 1997; Koerts et al., 1998; Rietjens et al., 1993). These studies indicate that approximately 80% of the administered dose is recovered as fluorinated urinary metabolites. Of these, approximately 50% of the administered dose is accounted for by monophenols, with 2-, 3-, and 4-fluorophenol present at a percent ratio of 33:20:47. The remainder of the urinary metabolites was composed of catechols, and presumably mercapturic acids, though these were not explicitly identified and quantified. The proportion of the administered dose recovered as catechols was not reported, but based on NMR spectra presented, the urinary concentration of 4-fluorocatechol appears to be approximately a third of the 3-fluorophenol concentration. In addition, a trace amount of 2-fluorocatechol was also detected. Direct quantitative comparison of the results for chloro- and fluorobenzene is difficult, since both the route of exposure (i.p. versus gavage, respectively) and the dose levels (4.5 mmol/kg versus 0.5 mmol/kg) are different between the studies. Considering the high dose used in the chlorobenzene study (4.5 mmol/kg), saturation of metabolic pathways may account for the lower proportion of the administered dose of chlorobenzene recovered as urinary metabolites. Consistent with this hypothesis is a second study of chlorobenzene metabolism in rats, in which approximately 32% of a 2 mmol/kg dose was recovered as urinary metabolites.

Overall, the pathways for metabolism of halobenzenes, including chlorobenzene and fluorobenzene are essentially identical. Although directly comparable quantitative studies could not be found for evaluation, the trend in the available data suggests that following comparable doses, the extent of flux through the various pathways is likely to be similar for both chloro- and fluorobenzene.

Figure 1. Metabolic Pathways for Monohalobenzenes.



(April 7, 2005)

Human Exposure Information

Fluorobenzene is received at a contract manufacturing site in isotanks that are delivered from ocean freight ports of entry into the U.S. It is pumped directly from the isotank into steel tanks in a diked area prior to use in production. This is a closed system. It is then pumped from the storage tank into the reactors for processing. This is also a closed system. At the end of the processing, the fluorobenzene has been consumed. During the purification of the product(s), any residual fluorobenzene is recovered to closed drums and then recycled into the process. Fluorobenzene has some vapor pressure, and so fugitive fluorobenzene vapors from the process are captured by an aqueous scrubber. That fluorobenzene is recovered and recycled. All hoses, lines, and fittings are inspected prior to use, and drained and dried after use.

During these operations, operators wear appropriate personal protective equipment (PPE) to protect themselves from splash and vapor. All waste and byproduct liquids (including water) that might contain fluorobenzene are captured and disposed of at regulated, off-site treatment, storage, and disposal facilities (TSDF's) or publicly owned treatment works (POTW's).

Potential exposure may occur during unloading and processing when operators are measuring the volumes in the tanks and process equipment. There is also the potential for exposure when any recovered fluorobenzene is drummed and transferred into the tank after recovery. At these times operators wear appropriate PPE. There is also the potential for exposure to fugitive emissions during line breaking operations or in the event of equipment failure. Operators and maintenance personnel wear appropriate PPE during line-breaking and maintenance operations to protect themselves from splash and vapor.

PPE consists at a minimum of safety glasses with side shields, goggles (or face shield), gloves, coveralls, workboots, and respirators with organic vapor/acid gas cartridges. Additional PPE may also be required, depending upon other issues relevant to the operation being carried out. Safety showers, eyewash stations, and Self Contained Breathing Apparatus (SCBA) are available in close proximity to the operations area.

The contract manufacturer has procedures, practices, and controls in place to manage the risk of exposure and no incidents have been reported to DuPont. DuPont practices Responsible Care® and assesses the ability of a potential contract manufacturer to safely handle fluorobenzene prior to commencing a commercial relationship. This assessment includes reviews and audits of PPE, safety equipment and procedures, structural integrity, and safety practices.

The DuPont Acceptable Exposure Limit (AEL) for fluorobenzene is 25 ppm (8- and 12-hour TWA). No other limits have been established. Air monitoring has been conducted on fluorobenzene and results are shown in the table below.

EXPOSURE DATA

No. of Results	Exposure period TWA (ppm)	8-hour TWA (ppm)	Min. of Results (ppm)	Max of Results (ppm)
19	0.69	0.69	0.1	5.9

While DuPont handles fluorobenzene as a closed system intermediate, DuPont cannot guarantee that all fluorobenzene users maintain the same level of controls. This robust summary contains the developmental toxicity data for the structurally related compound, chlorobenzene to meet the developmental toxicity endpoints.

References for Summary:

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(April 7, 2005)

TEST PLAN FOR FLUOROBENZENE

Fluorobenzene **CAS No. 462-06-6**	**Data Available**	**Data Acceptable**	**Testing Required**
Study	**Y/N**	**Y/N**	**Y/N**
PHYSICAL/CHEMICAL CHARACTERISTICS			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
ENVIRONMENTAL FATE			
Photodegradation	Y	Y	N
Stability in Water	Y	Y	N
Transport (Fugacity)	Y	Y	N
Biodegradation	Y	Y	N
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y*	Y	N
Acute Toxicity to Aquatic Plants	Y**	Y	N
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y	Y	N
Developmental Toxicity	Y**	Y	N
Reproductive Toxicity	Y**	Y	N
Genetic Toxicity Gene Mutations (in bacterial cells)	Y	Y	N
Genetic Toxicity Chromosomal Aberrations (in *in vivo* micronucleus test)	Y	Y	N

* 24-hour data were available for the test chemical and 48-hour data were available for an analog chemical.

** Data were available on an analog chemical.

ROBUST SUMMARY FOR FLUOROBENZENE

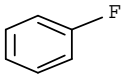
Existing published and unpublished data were collected and scientifically evaluated to determine the best possible study or studies to be summarized for each required endpoint. In the spirit of this voluntary program, other data of equal or lesser quality are not summarized, but are listed as additional references at the end of each appropriate section, with a statement to reflect the reason why these studies were not summarized.

1.0 Substance Information

CAS Number: 462-06-6

Chemical Name: Benzene, fluoro-

Structural Formula:



Other Names: Fluorobenzene
Monofluorobenzene
Phenyl fluoride

Exposure Limits: DuPont Acceptable Exposure Limit (AEL): 25 ppm (8- and 12-hour TWA)

2.0 Physical/Chemical Properties

2.1 Melting Point/Freezing Point

Value:	-40°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Budavari, S. et al. (1996). The Merck Index, 12th ed., p. 4212, Merck and Co., Inc., Rahway, NJ.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point:

Lewis, R. J. Sr. (1997). Hawley's Condensed Chemical Dictionary, 13th ed., p. 509, John Wiley & Sons, Inc., New York.

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Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 12th ed., Vol. II, p. 1796, John Wiley & Sons, Inc., New York.

Supporting Data: Chlorobenzene

Value:	-45.2°C
Decomposition:	No Data
Sublimation:	No Data
Pressure:	No Data
Method:	OECD Guideline 102
GLP:	No
Reference:	Solutia Inc. (2001). Material Safety Data Sheet (August 30) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Not assignable because limited study information was available.

2.2 Boiling Point

Value:	84.73°C @ 760 mm Hg 200°C @ 13 atmospheres 275°C @ 38 atmospheres
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Budavari, S. et al. (1996). The Merck Index, 12th ed., p. 4212, Merck and Co., Inc., Rahway, NJ.
Reliability:	Not assignable because limited study information was available.

Additional References for Boiling Point:

Lewis, R. J. Sr. (1997). Hawley's Condensed Chemical Dictionary, 13th ed., p. 509, John Wiley & Sons, Inc., New York.

Lide, D. R. (2001-2002). CRC Handbook of Chemistry and Physics, 82nd ed., p. 3-48, CRC Press, Boca Raton, FL.

DuPont Co. (1993). Material Safety Data Sheet No. DU002805 (March 9).

Weast, R. C. and M. J. Astle (eds.) (1989). CRC Handbook of Data on Organic Compounds, Vol. I, p. 175, CRC Press, Inc., Boca Raton, FL (cited in NTP Chemical Repository (1991). Radian Corporation August 29 accessed on http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002).

CHRIS (Hazmat data from the US Coast Guard) (CH-00000686).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 12th ed., Vol II, p. 1796, John Wiley & Sons, Inc., New York.

Supporting Data: Chlorobenzene

Value:	132.1°C
Decomposition:	No
Pressure:	1013 hPa
Method:	OECD Guideline 103
GLP:	No
Reference:	Solutia Inc. (2001). Material Safety Data Sheet (August 30) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Valid with restrictions

2.3 Density

Value:	1.024 g/mL
Temperature:	No Data
Method:	No Data
GLP:	Unknown
Results:	No additional data.
Reference:	Budavari, S. et al. (1996). The Merck Index, 12th ed., p. 4212, Merck and Co., Inc., Rahway, NJ.
Reliability:	Not assignable because limited study information was available.

Additional References for Density:

Lewis, R. J. Sr. (1997). Hawley's Condensed Chemical Dictionary, 13th ed., p. 509, John Wiley & Sons, Inc., New York.

Lide, D. R. (2001-2002). CRC Handbook of Chemistry and Physics, 82nd ed., p. 3-48, CRC Press, Boca Raton, FL.

DuPont Co. (1993). Material Safety Data Sheet No. DU002805 (March 9).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 12th ed., Vol. II, p. 1796, John Wiley & Sons, Inc., New York.

Lenga, R. E. (1985). The Sigma-Aldrich Library of Chemical Safety Data, Ed. 1, p. 953, Aldrich Chemical Co., Inc., Milwaukee, WI (cited in NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002).

Aldrich Chemical Co. (1988). Aldrich Catalog/Handbook of Fine Chemicals, p. 750, Aldrich Chemical Co., Inc., Milwaukee, WI (cited in NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002).

CHRIS (Hazmat data from the US Coast Guard) (CH-00000686).

Supporting Data: Chlorobenzene

Value:	1.107 (relative density)
Temperature:	4ºC
Method:	OECD Guideline 109
GLP:	No
Results:	No additional data.
Reference:	Solutia Inc. (2001). Material Safety Data Sheet (August 30) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Valid with restrictions

2.4 Vapor Pressure

Value:	60 mm Hg @ 19.6°C 100 mm Hg @ 30.4°C 1 mm Hg @ -43.4°C

	5 mm Hg @ -22.8°C 10 mm Hg @ -12.4°C 20 mm Hg @ -1.2°C 40 mm Hg @ 11.5°C 200 mm Hg @ 47.2°C 400 mm Hg @ 65.7°C 760 mm Hg @ 84.7°C
Temperature:	See above
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Stull, D. R. (1947). Ind. Eng. Chem., 39(4):523 (cited in NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002).
Reliability:	Not assignable because limited study information was available.

Additional References for Vapor Pressure:

Lide, D. R. (2001-2002). CRC Handbook of Chemistry and Physics, 82nd ed., p. 3-48, CRC Press, Boca Raton, FL.

DuPont Co. (1993). Material Safety Data Sheet No. DU002805 (March 9).

Dreisbach, R. R. (1961). Physical Properties of Chemical Compounds, Vol. III, American Chemical Society, Washington, DC (IS-0008277).

Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation, Hemisphere Publ. Corp., New York (EF-0013126).

Supporting Data: Chlorobenzene

Value:	11.7 hPa
Temperature:	20ºC
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Auer-Technikum, Auerges. MbH Berlin, 11. Ausgabe 1985 (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Valid with restrictions

2.5 Partition Coefficient (log Kow)

Value:	2.27
Temperature:	No Data
Method:	Measured
GLP:	Unknown
Reference:	Fujita, T. and J. Owasa (1964). J. Amer. Chem. Soc., 86:5175 (IS-0008279 and EF-0013125).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Partition Coefficient (log Kow):

Liu, Z. T. et al. (1996). Bull. Environ. Contam. Toxicol., 57:421-425.

Freed, V. H. et al. (1979). Environ. Health Perspect., 30:75-80.

Supporting Data: Chlorobenzene

Value:	2.64
Temperature:	25°C
Method:	Modeled. KOWWIN, v. 1.67, module of EPIWIN 3.11 (Syracuse Research Corporation). KOWWIN uses "fragment constant" methodologies to predict log P. In a "fragment constant" method, a structure is divided into fragments (atom or larger functional groups) and coefficient values of each fragment or group are summed together to yield the log P estimate.
GLP:	Not Applicable
Reference:	Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.
Reliability:	Estimated value based on accepted model.

2.6 Water Solubility

Value:	1.54 g/1000 g H_2O
Temperature:	20°C
pH/pKa:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Budavari, S. et al. (1996). The Merck Index, 12th ed., p. 4212, Merck and Co., Inc., Rahway, NJ.
Reliability:	Not assignable because limited study information was available.

Additional References for Water Solubility:

Lewis, R. J. Sr. (1997). Hawley's Condensed Chemical Dictionary, 13th ed.,

p. 509, John Wiley & Sons, Inc., New York.

DuPont Co. (1993). Material Safety Data Sheet No. DU002805 (March 9).

NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002.

Chiou, C. T. et al. (1977). Environ. Sci. Technol., 11:475-478 (EF-0013124).

Freed, V. H. et al. (1979). Environ. Health Perspect., 30:75-80.

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 12th ed., Vol. II, p. 1796, John Wiley & Sons, Inc., New York.

Supporting Data: Chlorobenzene

Value:	0.21 g/L
Temperature:	20°C
pH/pKa:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Bayer AG (1994). Safety Data Sheet (May 5) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Valid with restrictions

2.7 Flash Point

Value:	-15ºC
Method:	No Data
GLP:	Unknown
Reference:	National Fire Protection Association (1986). Fire Protection Guide on Hazardous Materials, 9th ed., p. 325M-54, National Fire Protection Association, Quincy, MA (cited in NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002)]
Reliability:	Not assignable because limited study information was available.

Additional References for Flash Point:

DuPont Co. (1993). Material Safety Data Sheet No. DU002805 (March 9).

Lewis, R. J., Sr. (2000). Sax's Dangerous Properties of Industrial Materials, 12th ed., Vol. II, p. 1796, John Wiley & Sons, Inc., New York.

Bretherick, L. (1985). Handbook of Reactive Chemical Hazards, 3rd ed., p. 577, 1732, Butterworths, London (cited in NTP Chemical Repository (1991). Radian Corporation August 29, http://ntp-db.niehs.nih.gov/NTP_Chem_H&S/NTP_Chem4/Radian462-06-6 accessed on June 25, 2002).

CHRIS (Hazmat data from the US Coast Guard) (CH-00000686).

Supporting Data: Chlorobenzene

Value:	ca. 27ºC
Method:	Closed cup, DIN 51755
GLP:	Unknown
Reference:	Bayer AG (1994). Safety Data Sheet (May 5) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003).
Reliability:	Valid with restrictions

2.8 Flammability: No Data.

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	No Data
Temperature:	25ºC
Direct Photolysis:	No Data
Indirect Photolysis:	Rate constant obtained at 25ºC or at room temperature was 0.69×10^{-12}. Half-life (23.3 days) calculated using an average atmospheric OH concentration of 5×10^5 molecule/cm^3.
Breakdown Products:	No Data
Method:	No Data
GLP:	Not Applicable
Reference:	Atkinson, R. (1989). J. Phy. Chem. Ref. Data, Monograph 1 (EF-0013127).
Reliability:	High based on accepted experimental methodology.

Concentration:	No Data
Temperature:	No Data
Direct Photolysis:	No Data
Indirect Photolysis:	Will be subject to radical reactions in surface waters.

Breakdown Products:	No Data
Method:	No Data
GLP:	Not Applicable
Reference:	Mill, T. 2000. "Photoreactions in surface waters", Ch. 15 In: R.S. Boethling and D. Mackay, Handbook of Property Estimation Methods for Chemicals, Lewis Publ., Boca Raton, FL.
Reliability:	High based on behavior of analogs.

Additional References for Photodegradation: None Found.

3.2 Stability in Water

Concentration:	Nominal test concentration = 504 µg/mL Actual concentrations upon dosing as determined by HPLC/UV ranged from 453-533 µg/mL.
Half-life:	Not Applicable
% Hydrolyzed:	Hydrolytically stable in pH 4, 7, and 9 buffer systems at 50±1 °C, as evidenced by ≥ 93% recovery for the Day 5 test system samples as a percentage of the Day 0 test system concentrations over all pHs. Mean of the Day 5 concentrations as percent of the Day 0 concentrations for pH 4, 7, and 9 were 102, 100, and 93%. Hydrolysis will not be a route of degradation in the environment.
Method:	The procedures used in the test were based on the recommendations of the following guidelines: OECD Guideline 111, US EPA Pesticide Assessment Guideline 161-1, and SETAC EUROPE (1995) "Procedures for assessing environmental fate and ecotoxicity of pesticides". The test substance was prepared in buffer solutions of pH 4, 7, and 9 and were maintained for 5 days at 50±1 °C. These solutions were then analyzed by HPLC\UV for the test substance.
GLP:	Yes
Reference:	DuPont Co. (2005). Unpublished Data, DuPont Study No. DuPont-15564, ABC Study No. 49189, "Hydrolysis of Fluorobenzene in Buffer Solutions of pH 4, 7, and 9" (February 23).
Reliability:	High because a scientifically defensible or guideline method was used.

Concentration: No Data
Half-life: No Data
% Hydrolyzed: No Data
Method: The HYDROWIN v1.67 module of EPIWIN v3.05 (Syracuse Research Corporation) can not estimate a hydrolysis rate constant for this type of chemical structure. The prediction methodology was developed for the U.S. Environmental Protection Agency and is outlined in Mill et al., 1987.
GLP: Not Applicable
Reference: Mill, T. et al. (1987). "Environmental Fate and Exposure Studies Development of a PC-SAR for Hydrolysis: Esters, Alkyl Halides and Epoxides," EPA Contract No. 68-02-4254, SRI International, Menlo Park, CA.
Reliability: Not Applicable.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity)

Media: Air, Water, Soil, and Sediments

Distributions:

Compartment	% of total distribution	½ life (hours) (advection + reaction)
Air	40.9	558
Water	44	900
Soil	14.8	1800
Sediment	0.245	8100

Adsorption Coefficient: Log Koc = 1.88
Desorption: No Data
Volatility: Henry's Law Constant = 0.0063 atm-m^3/mole
Method: Modeled. EPIWIN 3.11

Henry's Law Constant - HENRYWIN v3.10 module of EPIWIN v3.05 (Syracuse Research Corporation). Henry's Law Constant (HLC) is estimated by two separate methods that yield two separate estimates. The first method is the bond contribution method and the second is the group contribution method. The bond contribution method is able to estimate many more types of structures; however, the group method estimate is usually preferred (but not always) when all fragment values are available.

Log Koc – Calculated from log Kow by the Mackay Level III fugacity model incorporated into EPIWIN v3.05 (Syracuse Research Corporation).

Environmental Distribution - Mackay Level III fugacity model, in EPIWIN v3.05 (Syracuse Research Corporation). Emissions (1000 kg/hr) to air, water, and soil compartments.

Parameter Values:
Molecular Wt: 96.11
Henry's LC : 0.0063 atm-m^3/mole (Experimental)
Vapor Press : 71.7 mm Hg (MPBPWIN program)
Log Kow : 2.27 (Experimental)
Soil Koc : 76.3 (calc by model)

GLP: Not Applicable

Reference: HENRYWIN –

Hine, J. and P. K. Mookerjee (1975). J. Org. Chem., 40(3):292-298.

Meylan, W. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-1293.

Fugacity - The methodology and programming for the Level III fugacity model incorporated into EPIWIN v3.05 (Syracuse Research Corporation) were developed by Dr. Donald MacKay and coworkers and are detailed in:

Mackay, D. (1991). Multimedia Environmental Models: The Fugacity Approach, pp. 67-183, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Reliability: Estimated values based on accepted models.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation

Value:

Linear Model Prediction:	0.0199 – Does not biodegrade fast
Non-Linear Model Prediction:	0.0008 – Does not biodegrade fast

Ultimate Biodegradation Timeframe:	2.60 – weeks to months; corresponds to an estimated half-life of 37.5 days
Primary Biodegradation Timeframe:	3.73 – days to weeks
MITI Linear Model Prediction:	0.4847 - Does not readily biodegrade
MITI Non-Linear Model Prediction:	0.0257 – Does not readily biodegrade

Breakdown Products:	No Data
Method:	Modeled. BIOWIN, v4.0 module of EPIWIN v3.05 (Syracuse Research Corporation). BIOWIN estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental microorganisms. Estimates are based upon fragment constants that were developed using multiple linear and non-linear regression analyses.
GLP:	Not Applicable
Reference:	Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-65. Howard, P. H. et al. (1992). Environ. Toxicol. Chem., 11:593-603. Howard, P. H. et al. (1987). Environ. Toxicol. Chem., 6:1-10. Tunkel, J. et al. (2000). Predicting Ready Biodegradability in the MITI Test. Environ. Toxicol. Chem., accepted for publication.
Reliability:	Estimated value based on accepted model.

Additional Reference for Biodegradation:

Testing equivalent to a Modified MITI Test 301C indicates that fluorobenzene is not readily biodegradable.

Urano, K. and Z. Kato (1986). J. Hazard. Mater., 13(2):147-159 (CA105:65719x).

Results from environmental enrichment studies indicate that fluorobenzene has the potential to be inherently biodegradable. Microbial mineralization of

fluorobenzene will occur in less than four months, based on enrichments from sediments near industrial outfalls in northern Portugal.

Carvalho et al. (2002). Appl. Environ. Microbiol., 68(1), 102-105.

3.5 Bioconcentration

Value:	BCF: 11.17
Method:	Modeled. BCFWIN v2.4 module of EPINWIN v3.05 (Syracuse Research Corporation). BCFWIN estimates the bioconcentration factor (BCF) of an organic compound using the compound's log octanol-water partition coefficient (Kow) with correction factors based on molecular fragments.
GLP:	Not Applicable
Reference:	"Improved Method for Estimating Bioconcentration Factor (BCF) from Octanol-Water Partition Coefficient", SRC TR-97-006 (2nd Update), July 22, 1997; prepared for Robert S. Boethling, EPA-OPPT, Washington, DC, Contract No. 68-D5-0012; prepared by William M. Meylan, Philip H. Howard, Dallas Aronson, Heather Printup, and Sybil Gouchie, Syracuse Research Corp.
Reliability:	Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-Hour LC_{50}**
Species:	*Pimephales promelas*, fathead minnows
Value:	210 mg/L (95% confidence limits, 190-230 mg/L).
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The test material, prepared as a 1 mg/mL stock solution in laboratory distilled water, was placed into 5-L glass aquaria and diluted with laboratory well water to yield the desired nominal exposure concentrations in 4-L final volumes. An identical vessel, containing only laboratory well water, was designated as the control. Ten juvenile, unsexed fathead minnows, 2.0 cm mean standard length and 0.074 g mean wet weight, were randomly placed in each test vessel, 1 test vessel per concentration. Concentrations tested included 100, 133, 178,

237, 316, 422, 562.5, 750, and 1000 mg/L. Fish were not fed for approximately 48 hours prior to or during the exposure. The test solutions were not aerated, and temperature was maintained between 21.8 and 22.1ºC. Photoperiod was maintained at 16 hours light:8 hours dark. Mortality counts and observations were made approximately every 24 hours during the 96-hour exposure period.

Dissolved oxygen and pH were measured in the water control and in the low, medium, and high test concentrations (100, 316, and 1000 mg/L, respectively) at the beginning of the test, at 24-hour intervals during the 96-hour exposure period, or if total mortality occurred in a test concentration (72 hours at 316 mg/L, 20 minutes after test initiation at 1000 mg/L). Total alkalinity, hardness (EDTA), and conductivity were measured at the beginning of the test in the water control.

Concentration data were not transformed and the 96-hour LC_{50} and confidence intervals were calculated by probit analysis.

GLP: Yes

Test Substance: Fluorobenzene, purity >99%

Results: Control water alkalinity, hardness, and conductivity at 0 hours were 81mg/L as $CaCO_3$, 93 mg/L as $CaCO_3$, and 171 µmhos/cm, respectively. Dissolved oxygen concentrations and pH throughout the test ranged from 6.9-8.8 mg/L and 7.9-8.1, respectively.

The observed mortality at 96 hours was 0, 0, 0, 10, 90, 100, 90, 100, 100, and 100% for the 0, 100, 133, 178, 237, 316, 422, 562.5, 750, and 1000 mg/L concentrations, respectively.

Clinical signs observed on some fish at 100 mg/L and greater included erratic swimming, darkening in color, swimming at the surface, gasping for air, hyperactive, lying on the bottom, lethargy, moribundity, partial loss of equilibrium, rapid respiration, and blood stain at the gill area 5 to 10 minutes after the test was initiated. At 750 and 1000 mg/L, all the fish died 20 minutes after the test was initiated.

Reference: DuPont Co. (1986). Unpublished Data, Haskell Laboratory Report No. 376-86, "Static Acute 96-Hour LC_{50} of Fluorobenzene to Fathead Minnows" (June 28).

Chromey, N. C. et al. (1992). J. Am. Coll. Toxicol.,

	11(6):673-674.
Reliability:	Medium because a suboptimal study design with nominal concentrations was used for testing.
Type:	**48-hour LC_{50}**
Species:	*Carassias auratus*, goldfish
Value:	log1/LC_{50} value = 4.48 mol/L (430.5 mg/L)
Method:	The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline (1984). Directive 203. *Carassias auratus* were tested for 48-hours using a static-renewal method with test solution renewal at 12-hour intervals. Four fish (approximately 3.5 g weight and 4.0 cm length) were tested in 6-L glass beakers containing 4-L of test solution. Four to six concentrations were tested with 2 replicates of each concentration. Actual concentrations were not reported. Fish were not fed during exposure. The photoperiod consisted of 16-hours of light and 8-hours of darkness. Water temperature, dissolved oxygen, pH, and hardness were recorded. LC_{50} value was calculated using probit analysis.
GLP:	Unknown
Test Substance:	Fluorobenzene, purity ≥ 95%
Results:	Conditions of experimental water were temperature: 20±1ºC, dissolved oxygen: 8.2±0.5 mg/L, pH: 7.5±0.3, hardness (as $CaCO_3$): 110±10 mg/L.
Reference:	Liu, Z. T. et al. (1996). Bull. Environ. Contam. Toxicol., 57:421-425.
Reliability:	Medium because a suboptimal study design with nominal concentrations was used for testing.
Type:	**96-hour LC_{50}**
Species:	Fish
Value:	47.2 mg/L (log_{10} Kow of 2.19)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Fluorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution

Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

Reliability: Estimated value based on accepted model.

Supporting Data

Type: **96-hour LC_{50}**

Species: *Salmo gairdneri*, rainbow trout

Value: 10.4 mg/L

Method: The procedures used in the test were based on the recommendations of the following guideline: OECD Guideline 203.

Rainbow trout with a total length of 5±1 cm were used in the test. Light was provided with a daily photoperiod of 12 hours. Fish were not fed 24 hours prior to initiation of the test.

Glass tanks were filled with 18 L of reconstituted water. Tanks were covered with specially fitted glass plates assuring aeration and free exchange of the atmosphere. Test water was checked to verify that dissolved oxygen concentration, temperature, and pH were within specification limits. Test material was added to separate vessels at 0.03, 1.8, 3.2, 5.8, 10, 18, 32, 58, or 100 mg/L. A control vessel was also used. The contents of the tank were mixed with a mechanical stirrer. Air was continuously bubbled into the test medium through "flow out stones" directly connected to the aerator with PVC tubes. Aeration was necessary because O_2 concentration dropped below 60% of saturation within 6-8 hours. Fish were added to the tanks 2 hours later. Fish were inspected at 0, 24, 48, and 72 hours after study initiation. Dead fish were removed from the tanks and surviving fish were observed for illness.

LC_{50} values were obtained via probit analysis.

Samples of test medium were taken at the beginning, after 48 hours, and at the end of the test and were analyzed via GC-ECD. The first analytical tests were unsatisfactory because of high losses of test material during the storage time of the samples. Therefore, vessels were prepared containing concentrations equal to the LC_0, LC_{50}, and LC_{100} in the test. Samples were analyzed immediately.

GLP: Yes

Test Substance: Chlorobenzene, purity not reported

Results:	Analytical measurements of test concentration in the vessels indicated that only 0.26-2.2% of test material was recovered. Results of the second test revealed 10 (erroneous), 53, 3.5, and 2.4% recovery of a 5.8 mg/L nominal concentration at 0, 2, 6, and 20 hours; and 105, 60, 24, and 3.4% recovery of an 18 mg/L nominal concentration at 0, 2, 6, and 20 hours. Based on the % recovery at 20 hours, the average analytical concentrations over a 20-hour period for 5.8 (LC_0), 10.4 (LC_{50}), and 18 mg/L (LC_{100}) were 0.24, 0.3, and 0.61 mg/L. The value for the 10.4 mg/L concentration was calculated from an estimated average recovery of 2.9%. Results of the analytical tests showed that most of the test material was lost during the first 20 hours of the test. The fish that died either succumbed or showed abnormal behavior during the first few hours when the actual concentration was near the nominal concentration. Since it took 20 hours for all the fish to die, the data from this test were considered to be indicative of a 20-hour test. The nominal concentrations were considered to be more relevant for the evaluation of the test because all the fish that died were harmed by the high concentrations they were exposed to in the first hours of the test. No mortality was observed at 5.8 mg/L (nominal). In the 10 mg/L group (nominal), there was 40% mortality and at higher concentrations there was 100% mortality. All deaths occurred within 24 hours. Water was maintained anywhere from 91-100% saturation, a pH of 7.2-7.8, and a temperature of 14.1-15.5°C.
Reference:	Jones, W. (1990). "Investigation of the lethal effects of the test material chlorobenzene to the rainbow trout (static test) according to OECD Guideline 203" NATEC Project NA 89-9434 (April) (cited in Robust Summaries and Repository of Knowledge for CAS No. 108-90-7, http://www.epa.gov/chemrtk/viewsrch.htm accessed on March 4, 2003)
Reliability:	Medium because a suboptimal study design was used.
Type:	**96-hour LC_{50}**
Species:	Fish
Value:	20.9 mg/L (log_{10} Kow of 2.64)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Chlorobenzene
Results:	No additional data.

Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	15.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-2-fluorobenzene 15.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-3-fluorobenzene 15.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-4-fluorobenzene
Method:	Modeled
GLP:	Not Applicable
Test Substance:	1-Chloro-2-fluorobenzene 1-Chloro-3-fluorobenzene 1-Chloro-4-fluorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates

Type:	**24-hour EC_{50}**
Species:	*Daphnia*
Value:	7.37 mg/L (confidence limits, 6.42-8.40 mg/L)
Method:	The procedures used in the test were based on the recommendations of the following guidelines: OECD Guideline No. 202 and EEC Commission Directive 84/449, Test No. C-2. Dilution water was prepared by mixing Milli-Q and tap water in a basically 1:1 ratio, which was adjusted to attain a hardness of 150 mg/L ($CaCO_3$); total hardness of 130 mg/L ($CaCO_3$), pH of 8.2±0.5, and conductivity of 400±25 µs/cm. Stock solutions were prepared by adding the test chemical to

	the dilution water under mechanical stirring. Test solutions were obtained by diluting the stock solutions. The concentration of the test solutions was determined at the beginning and end of the experiment via high performance liquid chromatography. Ten daphnids, < 24 hours old, were subdivided into 2 replicates per concentration. At least 5 concentrations and a control were tested. The tests were performed in closed bottles filled to the top in order to avoid loss of chemical due to volatilization. The temperature was 20±1ºC and the photoperiod was 16 hours light, 8 hours dark. The test system was static. Test concentrations were not reported. Immobility was the endpoint evaluated. The raw data were analyzed by probit analysis. Estimates were obtained for the 24-hour EC_{50} as well as the slope of the concentration effect curve with their respective confidence intervals.
GLP:	Unknown
Test Substance:	Fluorobenzene, purity 99%
Results:	The slope of the dose response curve was 10.8 (confidence limits, 5.3-16.4). The number of immobilized daphnids was not reported. Dissolved oxygen was not reported.
Reference:	Tosato, M. L. et al. (1993). Sci. Total. Environ., Suppl. (1/2):1479-1490.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	51.3 mg/L (log_{10} Kow of 2.19)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Fluorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Supporting Data	
Type:	**24-hour EC_{50}**
Species:	*Daphnia*
Value:	4.3 mg/L (95% confidence limits, 3.25-5.7 mg/L)
Method:	The procedures used in the test were based on the recommendations of the following guidelines: AFNOR, Norme Experimentale N. F. T. 90-301 (1974). The AFNOR test was used to define the 24-hour IC_{50}, the immobilization concentration for 50% of the animals at 24 hours. The concentration of chlorobenzene in the test waters was determined at the beginning and end of the experiment and analyzed via gas chromatography. The tests were carried out in closed systems. Effective concentrations (EC_{50}) for immobilization data were extrapolated from empirical curves fitted by eye on log-probability paper and not elaborated, being very close to the concentration with 0 and 100% immobilized animals.
GLP:	No Data
Test Substances:	Chlorobenzene, pure compound analytical grade
Results:	Results are reported as nominal concentrations since the differences between the observed and expected concentrations did not exceed 10% of the initial value. Repeated measurements of pH and O_2 in the test solutions did not show fluctuations higher than 10%. No other details on water chemistry parameters were reported.
Reference:	Calamari, D. et al. (1983). Chemosphere, 12(2):253-262.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	23.4 mg/L ($\log_{10}$ Kow of 2.64)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Chlorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).

(April 7, 2005)

Reliability:	Estimated value based on accepted model.
Type:	**48-hour EC_{50}**
Species:	*Daphnia*
Value:	2.28 mg/L (95% confidence limits, 1.86 – 2.55 mg/L): 1-Chloro-2-fluorobenzene 3.64 mg/L (95% confidence limits, 3.19 – 4.19 mg/L): 1-Chloro-3-fluorobenzene 1.70 mg/L (95% confidence limits, 1.24 – 2.13 mg/L): 1-Chloro-4-fluorobenzene
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. Test solutions were obtained from saturated stock solutions prepared by adding excess chemical to dilution water and slowly stirring for at least 20 hours. The dilution water was bottled natural mineral water diluted with distilled water and aerated to reach oxygen saturation. The water had a pH of 8.3±1, conductivity of 311±33 µs/cm, and hardness of 160±20 mg/L as $CaCO_3$. Daphnia were < 24 hours old. Tests were run in a thermostatic room at 21.5ºC with a 16:8 hour light:dark photoperiod. A minimum of 5 concentrations plus the control were tested, spaced by a geometric factor of 1.2 to 1.8. Twenty animals, divided into 4 replicates, were exposed to each concentration in 50 mL glass flasks. The flasks were completed filled and were closed with glass stoppers. The test system was static. The endpoint evaluated was immobility. At the beginning and end of the test, temperature, pH, dissolved oxygen, and conductivity were measured in the control and a minimum of 2 other concentrations. Hardness was measured in the control and highest concentration. Test substance concentrations were confirmed by gas chromatography. Toxicity data were analyzed using the probit analysis.
GLP:	No Data
Test Substances:	1-Chloro-2-fluorobenzene, purity >98% 1-Chloro-3-fluorobenzene, purity >98% 1-Chloro-4-fluorobenzene, purity >98%
Results:	At the end of the test, immobilization in the control was

	≤10%. The number of immobilized daphnids was not reported for the test concentrations. Based on analytical measurements, test substance loss during each study was < 20% for all test substances and test concentrations except for 1-chloro-3-fluorobenzene. Dissolved oxygen was maintained above 75% saturation. No other details on water chemistry parameters were reported.
Reference:	Marchini, S. et al. (1999). Environ. Toxicol. Chem., 18(2):2759-2766.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**48-hour EC_{50}**
Species:	Daphnid
Value:	17.8 mg/L (log_{10} Kow of 2.84): 1-Chloro-2-fluorobenzene 17.8 mg/L (log_{10} Kow of 2.84): 1-Chloro-3-fluorobenzene 17.8 mg/L (log_{10} Kow of 2.84): 1-Chloro-4-fluorobenzene
Method:	Modeled
GLP:	Not Applicable
Test Substance:	1-Chloro-2-fluorobenzene 1-Chloro-3-fluorobenzene 1-Chloro-4-fluorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	32.4 mg/L (log_{10} Kow of 2.19)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Fluorobenzene
Results:	No additional data.

Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Supporting Data

Type:	**96-hour EC_{50}**
Species:	*Selenastrum capricornutum*, algae
Value:	12.5 mg/L
Method:	The procedures used in the test were based on a modification of the Algal Assay Procedure Bottle Test (AAPBT) (USEPA) batch test. The procedure permits maintenance of a constant concentration of toxicant in the culture and calculation of the concentration in the culture medium at equilibrium, on the basis of physical characteristics of the chemical. Algal cultures were set up in 2-L spherical flasks closed by screw cap with both silicone rubber and teflon gaskets. The caps were pierced by a stainless steel needle dipping in to the culture medium. Sampling for measurement of algal growth and toxicant concentration was made through the needle by means of a syringe. The volume of the culture medium was 100 mL. The measured medium to flask volume ratio (0.047) was low enough to avoid notable carbon dioxide deficiency. Culture medium and test conditions were similar to the AAPBT, with the exception that the temperature was 20±1ºC. Stock solutions were prepared as follows: an amount of the chemical 10 times higher than the saturation solubility was added to distilled water in a closed vessel, stirred for 48 hours, and decanted for 24 hours. The supernatant was filtered through paper-filters, and the concentration was measured. Final solutions were made by adding 10 mL of stock culture medium to different amounts of toxicant stock solution. Solutions were diluted to 100 mL with distilled water and quickly transferred into the culture flask. Capped flasks were shaken for 24 hours at 20ºC to let vapor and liquid phases equilibrate. The algal inoculum was added, after reaching equilibrium, at a starting cell concentration of $5x10^6$ cells/L.

Test medium and test conditions were reported to be similar to those presented in US EPA (1971). Algal Assay Procedure – Bottle Test, National Eutrophication Research Program, Corvallis, except that temperature was maintained at 20±1ºC.

The concentration of the test substance was measured by gas chromatographic analysis after the 24 hour equilibrium and 48 and 96 hours after the inoculum was added.

Algal growth was measured at 24, 48, and 96 hours by *in vivo* fluorescence. Results were expressed as a percentage of growth in the control culture and graphically elaborated for the evaluation of the EC_{50}.

GLP: No Data

Test Substance: Chlorobenzene, purity not reported

Results: Initial concentrations could not be measured due to the high volatility of the test material. Within a few minutes of adding the test material to the flasks the concentration was very low compared to theoretical values. Therefore, the initial concentrations calculated from the dilution of the titrated stock solutions were assumed to be the initial concentrations. Equilibrium concentrations were calculated as the mean of the analytical concentrations in samples taken after the equilibrium period and 48 and 96 hours. For initial concentrations of 31.6, 63.2, 94.8, 126.4, 158.0, 221.2, and 284.4 mg/L, equilibrium concentrations of 6.5, 14.3, 23.3, 29.6, 37.8, 45.0, and 63.0 were determined, respectively. The mean initial concentration/equilibrium concentration was 4.5±0.3. The calculated Henry's constant (0.16) was fairly close to the reported value of 0.11, confirming the validity of the method for prediction of concentrations at equilibrium.

After a 24-hour equilibrium period, the concentration of the test material in the culture medium remained almost constant. Differences in the values obtained at equilibrium and after 48 and 96 hours were within the range of acceptable analytical variability. The cell concentration per test concentration at each measurement interval was not reported.

The 96-hour EC_{50} value calculated for chlorobenzene inhibition of algal fluorescence was 12.5 mg/L. The 95% confidence interval was not reported. The maximum tested

	concentration that produced no effect was <6.8 mg/L and the minimum concentration that was 100% effective was 46.3 mg/L.
Reference:	Galassi, S. and M. Vighi (1981). Chemosphere, 10(10):1123-1126.
Reliability:	High because a scientifically defensible or guideline method was used.

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	15.2 mg/L (log_{10} Kow of 2.64)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	Chlorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Type:	**96-hour EC_{50}**
Species:	Green algae
Value:	11.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-2-fluorobenzene 11.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-3-fluorobenzene 11.7 mg/L (log_{10} Kow of 2.84): 1-Chloro-4-fluorobenzene
Method:	Modeled
GLP:	Not Applicable
Test Substance:	1-Chloro-2-fluorobenzene 1-Chloro-3-fluorobenzene 1-Chloro-4-fluorobenzene
Results:	No additional data.
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral ALD**
Species/Strain:	Male rats/Crl:CD®(SD)BR
Value:	11,000 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. The test substance was suspended in corn oil and administered to 1 rat per dose level by intragastric intubation. Dose levels used in the study included 2300, 3300, 5000, 7500, and 11,000 mg/kg. Male rats were 7 weeks of age when received for the study and 8 weeks of age at test substance administration. Following administration of the test substance, rats were observed for clinical signs of toxicity. Surviving rats were weighed and observed daily until signs of toxicity subsided, and then at least 3 times a week throughout the 14-day observation period. Pathological examinations were not performed.
GLP:	Yes
Test Substance:	Fluorobenzene, purity >99%
Results:	Mortality occurred in the 11,000 mg/kg dose group only. At this dose level, severe body weight loss (i.e., 11% of body weight) was observed 1 day after dosing. Clinical signs of toxicity included limpness, no righting reflex, rapid breathing, and clear discharge from both eyes. Death occurred within 2 days of dosing. At the non-lethal doses, slight to severe weight losses (i.e., 5-13% of body weight) were observed 1 day after dosing. There were no other common clinical signs of toxicity observed.
Reference:	DuPont Co. (1986). Unpublished Data, Haskell Laboratory Report No. 408-86, "Approximate Lethal Dose (ALD) of Fluorobenzene in Rats" (July 23). Chromey, N. C. et al. (1992). J. Am. Coll. Toxicol., 11(6):673-674.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Acute Oral Toxicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Azouz, W. M. et al. (1952). Biochem. J., 50:702-706 (CA46:5116d).

Eitingon, A. I. and I. P. Ulanova (1975). Gig. Tr. Prof. Zabol., (9):36-39 (CA84:26568a) (RTECS/DA0800000).

Hoechst AG (1969). Project No. 124/69 (cited in Anon. (1995). Toxikologische Bewertung, 126:1-13).

Type:	**Inhalation ALC**
Species/Strain:	Male rats/Crl:CD®(SD)BR
Exposure Time:	4 hours
Value:	6200 ppm
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Vapor atmospheres of fluorobenzene were generated by pumping the liquid test material into a 3-neck glass mixing flask. The flask was heated to 84-93ºC to facilitate evaporation. The flask temperature was controlled with a controller and was monitored with a thermocouple thermometer. Air introduced at the flask swept the fluorobenzene vapors through a glass dispersion funnel and into the exposure chamber. Additional dilution air was added to the vapor mixture prior to its entry into the chamber. The atmospheric concentration of the fluorobenzene was determined at approximately 30-minute intervals during each exposure by gas chromatography. During each exposure, chamber temperature, relative humidity, and chamber oxygen content were measured.

Each group of 6 rats was exposed nose-only for 4 continuous hours to 50, 520, 3700, 6200, or 10,000 ppm fluorobenzene. Rats were 8 weeks old and weighed between 229 and 269 grams on the day they were exposed.

Rats were weighed and observed prior to exposure. Group observation of clinical signs of toxicity were taken during each exposure and when rats were released from the restrainers after exposure. Surviving rats were weighed and observed daily for 14 days post-exposure, weekends and

	holidays included when warranted by the rats' condition. No pathological examination was conducted.
GLP:	Yes
Test Substance:	Fluorobenzene, purity >99%
Results:	Chamber temperature ranged from 21-24ºC, relative humidity ranged from 7-14%, and chamber oxygen ranged from 20-21%. Mortality of 0/6, 0/6, 0/6, 2/6, and 5/6 was observed in the 50, 520, 3700, 6200, 10,000 ppm groups, respectively. During or immediately after exposure, rats exposed to 50, 520, or 6200 ppm had red nasal or ocular discharges, effects common to rats under restraint. Rats exposed to 10,000 ppm had no response to sound during exposure. When released from restrainers after exposure, rats exposed to 3700 ppm and rats that survived exposure to 6200 and 10,000 ppm had rapid breathing, tremors, spasms, no righting reflex, and clear ocular discharge. During the post-exposure period, no significant weight loss or adverse clinical signs were observed in rats that survived exposure to fluorobenzene.
Reference:	DuPont Co. (1986). Unpublished Data, Haskell Laboratory Report No. 769-86, "Inhalation Approximate Lethal Concentration (ALC) of Fluorobenzene" (December 19). Chromey, N. C. et al. (1992). J. Am. Coll. Toxicol., 11(6):673-674.
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Acute Inhalation Toxicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Eitingon, A. I. and I. P. Ulanova (1975). Gig. Tr. Prof. Zabol., (9):36-39 (CA84:265 (RTECS/DA0800000).

Lapik, A. S. (1965). Izv. Sibirsk. Otd. Akad. Nauk S.S.S.R., Ser. Biol-Med. Nauk., (3):91-94 (CA64:20497f).

Type: **Dermal Toxicity:** No Data

(April 7, 2005)

Type:	**Dermal Irritation**
Species/Strain:	Female rabbits/New Zealand White
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. On the day prior to exposure, the hair of 6 rabbits was closely clipped to expose the back from the scapular to the lumbar region. The rabbits weighed from 3051 to 3611 g on the day of treatment. Each rabbit was placed in a stock which had been fitted with rubber sheeting. The rabbits remained in the stock throughout the exposure period and during that time did not have access to food or water. A 0.5 mL aliquot of fluorobenzene was applied directly to the test site beneath a 1-inch gauze square held in place with tape. The rubber sheeting was then wrapped around the animal and secured with clips to retard evaporation and to keep the test material in contact with the skin without undue pressure. Approximately 24 hours after treatment, the wrappings were removed. Excess test substance was washed from the rabbit's back. The skin was patted dry and the animals were returned to their cages. Approximately 25 and 48 hours after application of the test material, the test sites were examined for erythema, edema, and other evidence of dermal effects and were scored according to the Draize scale. Adjacent areas of the untreated skin were used for comparison. No pathology examinations were conducted.
GLP:	Yes
Test Substance:	Fluorobenzene, purity >99%
Results:	Fluorobenzene produced slight to mild erythema in 3 of 6 rabbits 25 hours after treatment. Mild to severe edema was observed in 2 rabbits. At 48 hours, slight to mild erythema was observed in 4 rabbits and slight to mild edema was noted in 3 rabbits. At 72 hours post-treatment, slight to mild erythema was noted in 4 of 6 rabbits and slight to mild edema was observed in 3 rabbits. No other dermal effects were noted. Fluorobenzene was considered a mild skin irritant.
Reference:	DuPont Co. (1986). Unpublished Data, Haskell Laboratory

	Report No. 548-86, "Skin Irritation Test in Rabbits of Fluorobenzene" (September 2).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for Dermal Irritation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Bagley, D. M. et al. (1996). Toxicol. *In Vitro*, 10:1-6.

Type:	**Dermal Sensitization**
Species/Strain:	Guinea Pig/strain not reported
Method:	No Data
GLP:	Unknown
Test Substance:	Fluorobenzene, purity not reported
Results:	Fluorobenzene does not induce sensitization in the skin of guinea pigs.
Reference:	BG Chemie (1993). Toxikol. Bewertung, No. 126 (August) (cited in Bayer, E. and G. Fleischhauer (1993). Chemosphere, 26(10):1789-1822).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Dermal Sensitization:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Hoechst AG (1969). Project No. 124/69 (cited in Anon. (1995). Toxikologische Bewertung, 126:1-13).

Type:	**Eye Irritation**
Species/Strain:	Male rabbits/New Zealand White
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. On the day of study initiation, the eyes of 2 rabbits were examined to ensure that no pre-existing corneal or conjunctival injury or irritation was present. The rabbits weighed 2587 and 3875 g on the day of treatment.

	A 0.01 mL aliquot of fluorobenzene was introduced into the lower conjunctival sac of the left eye of 2 rabbits. The right eyes served as controls. The treated and control eyes of 1 rabbit remained unwashed. Approximately 20 seconds after the test material was administered, both eyes of the remaining rabbit were rinsed for 1 minute with lukewarm water. Approximately 1 and 4 hours and 1, 2, and 3 days after treatment, the rabbits were examined for evidence of eye irritation. The washed eye was also examined 7 days after treatment. At each observation, the treated eyes were examined using illumination and magnification and scored for ocular reactions using the Draize scale. Fluorescein dye was used to evaluate corneal ulceration and irritation starting at the 24-hour observation and at each subsequent observation. Biomicroscopic examinations for corneal injury were conducted at the 24-hour observation period and each subsequent observation.
GLP:	Yes
Test Substance:	Fluorobenzene, purity >99%
Results:	Instillation of the undiluted liquid into the rabbit eye produced no corneal opacity, mild conjunctival redness with slight chemosis, and moderate blood-tinged discharge in the unwashed eye. Washing of the exposed eye produced more severe effects (slight corneal opacity, moderate conjunctival redness with mild chemosis, and copious blood-tinged discharge). The unwashed eye was normal by day 3 post-instillation and the washed eye was normal by day 7. Biomicroscopic and fluorescein staining evaluations were negative at all intervals for corneal injury. Fluorobenzene was considered a moderate eye irritant.
Reference:	DuPont Co. (1986). Unpublished Data, Report No. 420-86, "Eye Irritation Test in Rabbits of Fluorobenzene" (August 21).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional References for Eye Irritation:

Data from these additional sources indicate that fluorobenzene is a severe eye irritant. These studies were not chosen for detailed summarization because limited study information was available.

BG Chemie, Toxikol. Bewertung (1993). No. 126 (August) (cited in Bayer, E. and

G. Fleischhauer (1993). Chemosphere, 26(10):1789-1822).

Hoechst AG (1969). Project No. 124/69 (cited in Anon. (1995). Toxikologische Bewertung, 126:1-13).

5.2 Repeated Dose Toxicity

Type:	**28-Day Inhalation Study**
Species/Strain:	Rats/Sprague-Dawley CD
Sex/Number:	Male and female/5 per sex per group
Exposure Period:	28 consecutive days
Frequency of Treatment:	6 hours/day
Exposure Levels:	0, 0.4, 1.5, 6.0 mg/L (0, 94, 381, 1585 ppm)
Method:	The procedures used in the test were based on the recommendations of the following guidelines: Method B8, Annex V of the EEC Commission Directive 84/449/EEC and OECD Guideline 412.

With the exception of the 6-hour exposure, the rats were housed in groups of 5 by sex. At the start of treatment, male rats weighed 168-212 g and female rats weighed 136-188 g, and were approximately 7-8 weeks old.

For atmosphere generation, the test material was contained in glass flasks held in water baths and maintained at 20ºC. Compressed air was supplied by a compressor and was passed through a water trap and respiratory quality filters before it reached the system. The main air supply was fed through a tangential channel at the top of each chamber. A small amount of this air was diverted and bubbled through the test material before being ducted to the top of the exposure chamber. The control chamber received air only at a similar flow rate to the other chambers. The cylindrical exposure chambers had a volume of ~30 L. The concentration in the chambers was controlled by adjusting the flow rate of the air through the test material.

Each rat was individually restrained in a tapered, polycarbonate tube fitted onto a single tier of the exposure chamber. The animals' positions were rotated daily. Only the noses of the animals were exposed to the test atmosphere.

The temperature and relative humidity inside the exposure chambers were measured daily. Oxygen levels in the

chambers were measured weekly. The concentration of the test atmosphere was measured daily by high performance liquid chromatography.

Animals were continuously monitored during the exposure for any changes in appearance, respiratory, and behavioral patterns. Clinical observations were recorded prior to the start of exposure and on removal from the chambers. Body weights, food consumption, and water consumption were measured periodically throughout the study.

Home cage observations, open field, and functional observations were performed on the day prior to the start of dosing and subsequently on days 13 and 14, and 27 and 28 for males and females, respectively.

Hematological and blood chemistry investigations were performed on all animals prior to necropsy on day 29. Animals were not fasted prior to sampling. Urine samples were collected over a period of ~16 hours, by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection, but did not have access to food. The following hematology parameters were measured or calculated: hematocrit, hemoglobin, erythrocyte count, total leucocyte count, differential leucocyte count, platelet count, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration. Clotting time was assessed by Hepato Quick time using samples collected into sodium citrate solution. The following blood chemistry parameters were measured or calculated: blood urea, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glucose, and total bilirubin. The following parameters were measured on the freshly collected urine: volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, reducing substances, blood, and microscopic examination of sediment.

At study termination, all rats underwent a gross necropsy. Organ weights relative to brain and body weights were calculated for 9 organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes including epididymides). Representative samples of approximately 35 tissues were taken. These tissues included the adrenals,

aorta, bone and bone marrow, brain, cecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes, mammary gland, muscle, nasal cavity, esophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, stomach, testes with epididymides, thymus, thyroid/parathyroid, trachea, urinary bladder, and uterus. All preserved tissue sections from the control and high dose group were prepared, sectioned, and stained with hematoxylin and eosin for subsequent microscopic examinations. Lungs, any gross lesions, liver, and kidneys from animals in the low and intermediate dose groups were also examined.

Samples of sternum bone and teeth were taken from each animal and pooled per cage group for fluoride analysis.

Data were processed to give group mean values and standard deviations where appropriate. Absolute and relative organ weights, hematological, and blood chemistry data were analyzed by one-way analysis of variance incorporating F- max test for homogeneity of variance. Data showing heterogeneous variances were analyzed using Kruskal Wallis analysis of variance and Mann Whitney U-Test.

GLP: Yes

Test Substance: Fluorobenzene, purity not reported

Results: The mean achieved atmosphere concentrations were 0.37, 1.50, and 6.24 mg/L for the 0.4, 1.5, and 6.0 mg/L groups, respectively.

There were no deaths during the study. Incidents of red/brown staining of the external body surface and wetness of the fur were detected in all groups. The authors note that these were normal findings associated the restraint procedure and they were not indicative of toxicity. Hunched posture and piloerection were observed in the intermediate (1.50 mg/L) and high dose group (6.24 mg/L) animals. The 6.24 mg/L animals began showing these clinical signs on removal from the chamber from day 7 onwards. The incidence increased as the study progressed, and by day 24 all animals in this group were showing the signs both prior to exposure and on removal from the chamber. The 1.50 mg/L animals began showing these signs from day 21 onwards.

There was no evidence of significant neurotoxicity. No adverse effects on body weight gain, food consumption,

water consumption, hematology parameters, blood chemistry parameters, or urinalysis were detected. There were no treatment-related macroscopic abnormalities detected at necropsy.

A statistically significant increase in absolute and relative liver weight was detected in the 1.50 and 6.24 mg/L males, and relative liver weight was elevated in the 6.24 mg/L females. Kidney weight, relative to body weight, showed a statistically significant increase in the 6.24 mg/L males. No treatment-related effects were detected in the 0.37 mg/L groups.

Other effects of treatment were confined to adaptive liver changes (centribolular hepatocyte enlargement) and unique male rat hydrocarbon nephropathy. There was no associated evidence of hepatocellular degeneration and no associated inflammatory response. Although the adaptive liver changes extended into the low dose group (0.37 mg/L), the condition was not considered to be a toxicologically important adverse effect of treatment. In the kidneys, eosinophilic droplets were observed in the proximal tubular epithelium of male rats exposed to 1.50 and 6.24 mg/L. This condition was considered to be related to treatment even though the females were not affected. The authors note that eosinophilic droplet formation in the renal tubular epithelium is a typical consequence of hydrocarbon administration, and is peculiar to the male rat.

The incidence of the above mentioned effects in male rats is presented in the table below:

	Exposure Level (mg/L)			
	0	0.37	1.50	6.24
Kidneys: Eosinophilic droplets proximal tubular epithelium				
Absent	5	5	2	1
Minimal	0	0	3	3
Slight	0	0	0	1
Liver: Centrilobular hepatocyte enlargement				
Absent	5	3	2	1
Minimal	0	2	3	3
Slight	0	0	0	1

A substantial increase in fluoride levels was detected in the pooled teeth and sternum samples from all treatment groups. A clear dose response was apparent.

The NOAEL was considered to be 0.37 mg/L (94 ppm). Furthermore, the slight changes observed in physical condition were not indicative of serious damage to the health of the animals. There was, however, evidence of a treatment-related increase in fluoride concentration in bones and teeth of animals from all exposure groups.

Reference: Safepharm Labs. Ltd. (1993). Project No. 121/194, "28-Day Subacute (nose-only) Inhalation Toxicity Study in the Rat" (January 12) (cited in TSCA Fiche OTS0572061).

Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 277-92, "Two-Week Inhalation Toxicity Study in Rats with Fluorobenzene" (October 5).

Sellei, C. et al. (1953). Arch. Geschwulstforsch., 5:263-264 (CA49:13451d).

Nemeth, L. et al. (1957). Arch. Geschwulstforsch., 11:101-111 (CA54:3739c).

Supporting Data: Chlorobenzene

Type:	**13-Week Gavage Study**
Species/Strain:	Rats/Fischer 344 Mice/B6C3F1
Sex/Number:	Male and female/10 per sex per group
Exposure Period:	13 weeks
Frequency of Treatment:	5 days/week
Exposure Levels:	0, 60, 125, 250, 500, 750 mg/kg
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The animals were 6-8 weeks of age at study start. Animals were housed 5/cage. Dose solutions were prepared with corn oil every 7-12 days and stored in the dark at 4°C.

The animals were observed twice daily for mortality and morbundity. Clinical signs of toxicity were recorded daily and body weights and cage food consumptions were measured weekly.

During the final week of the study, rats and mice were placed in metabolism cages (1 rat/cage; 3-6 mice/cage) for the collection of 24-hour urine samples. Urine volume was measured, and pH, protein, glucose, ketones, bilirubin, and occult blood were assessed with reagent strips. Specific gravity and creatinine concentration were also measured. Samples of urine were applied to anion-exchange columns for separation of porphyrins, and the eluates were assayed for uroporphyrins and coproporphyrins and uroporphyrins.

Blood was taken from the orbital venous plexus of each animal and analyzed for hemoglobin content, packed cell volume, total and differential white blood cell count, red blood cell count, mean corpuscular volume, platelet count, and reticulocyte count.

At the end of the 13-week exposure period, animals were sacrificed and blood samples were obtained by cardiac puncture. Serum was analyzed for alkaline phosphatase, glutamic pyruvic transaminase, and gamma glutamyl transpeptidase activities, bilirubin, cholesterol, glucose, triglyceride, urea nitrogen, total protein, and globulin concentrations. Samples of liver were obtained and

homogenized. Total liver porphyrins were extracted and measured.

Body, lung, liver, heart, spleen, thymus, brain, kidney, testis ovary, and uterus weights were measured at sacrifice. All animals were given a gross examination. The following tissues, and all gross lesions, were removed, fixed, sectioned and stained: mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, gall bladder (mouse), prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, and mammary gland. Microscopic examinations occurred in the control, 250 mg/kg (mice only), 500 mg/kg, and 750 mg/kg groups, and from all animals that died during the study.

All clinical chemistry, hematology, and organ weight data were analyzed with Dunnett's multiple comparison test.

GLP: No Data

Test Substance: Chlorobenzene, purity >99.9%

Results: Survival was reduced in male and female rats at 500 and 750 mg/kg. Mortality of 1/10, 0/10, 0/10, 0/10, 4/10, and 9/10 occurred for the 0, 60, 125, 250, 500, and 750 mg/kg male rats, respectively. Mortality of 0/10, 1/10, 0/10, 0/10, 3/10, and 8/10 occurred for the 0, 60, 125, 250, 500, and 750 mg/kg male rats, respectively. Survival was reduced in male and female mice at 250, 500, and 750 mg/kg. Mortality of 0/10, 0/10, 0/10, 5/9, 10/10, and 10/10 occurred for the 0, 60, 125, 250, 500, and 750 mg/kg male mice, respectively. Mortality of 1/10, 0/10, 0/10, 4/10, 7/10, and 10/10 occurred for the 0, 60, 125, 250, 500, and 750 mg/kg female mice, respectively. Most of the deaths occurred in the later half of the study, except for the male mice in the 500 and 750 mg/kg groups.

Clinical signs of toxicity were not observed. Body weight gains were reduced in the male rats at 250 mg/kg and above, and in female rats at 500 mg/kg and above. Body weight gains were reduced in the male mice at 250 mg/kg and above, and in female rats at 500 mg/kg and above.

Slight increases in serum alkaline phosphatase and slight to moderate increases in serum gamma glutamyl transpeptidase activities were observed in the surviving female rats at 500 and 750 mg/kg. No alterations in serum chemistries were

observed in the mice. Consistent changes in hematological parameters were not observed in rats or mice. No abnormalities were observed in urine composition, although a mild polyuria was apparent in some of the higher-dose animals.

A slight increase in total liver porphyrin was observed in the surviving female rats at 500 and 750 mg/kg, and porphyrinuria was detected in male and female rats and in female mice at the higher doses.

Slight to marked increases in liver weights were observed in the rats and mice in a dose-related manner. Slight increases in kidney weights and decreases in spleen weights were also observed in male and female rats and female mice.

Pathologic examination revealed lesions in both sexes of the rats and mice at 250 mg/kg and greater in the liver (centrilobular hepatocellular degeneration and necrosis), kidney (mild to moderate vacuolar degeneration and focal coagulative necrosis of the proximal tubules), thymus (mild to severe lymphoid or myeloid depletion), bone marrow (minimal to moderate myeloid depletion), and spleen (minimal to mild lymphoid or myeloid depletion).

No toxic effects were observed at 125 mg/kg or less.

Reference: Kluwe, W. M. et al. (1985). J. Toxicol. Environ. Health, 15:745-767.

Reliability: High because a scientifically defensible or guideline method was used.

Supporting Data: Chlorobenzene

Type:	**13-Week Inhalation Study**
Species/Strain:	Rats/Charles River albino Dogs/Beagle
Sex/Number:	Male and female rats/15 per sex per group Male and female dogs/4 sex per group
Exposure Period:	13 weeks
Frequency of Treatment:	6 hours days/day, 5 days/week (excluding holidays)
Exposure Levels:	0, 0.75, 1.50, 2.00 mg/L
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Animals were exposed in specially constructed stainless steel

and glass inhalation chambers, each with a capacity of 8 m^3. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. Untreated control animals received exposure to clean air only. Vapors of undiluted test material were generated by passing a stream of clean, dry air through each test generator flask containing the test material. The resulting air and vapor mixtures were introduced into the exposure chambers at the top center and exhausted through the bottom of each chamber. A stream of additional conditioned air was supplied to each chamber at a rate of 700 L/min. Generator air flow rates were measured with rotameters. The temperature and barometric pressure of each chamber were measured daily. Chamber concentrations were periodically measured and analyzed via gas chromatography.

The animals were observed daily for mortality and abnormal reactions. Body weights were measured weekly.

Hematology studies, including hemoglobin concentration, erythrocyte count, hematocrit value, and total and differential leukocyte counts were conducted on samples from all dogs obtained before the first exposure and all surviving dogs at 39 and 88 days of the test period. The same blood studies were conducted on samples obtained from 5 male and 5 female rats from the control and 2 mg/L groups at day 39 and 91. Special bleedings were performed on several dogs that were in a moribund condition.

Clinical chemistry studies were conducted on the samples obtained from the same animals used in the hematology studies. Blood urea nitrogen (BUN), serum alkaline phospatase activity (SAP), serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) (dogs only), and fasting blood glucose were determined.

Animals were kept in metabolism cages for 24 hours prior to the above bleedings, and urine samples were collected. These samples were examined for albumin, glucose, microscopic elements, pH, and specific gravity.

At the time of sacrifice, all animals underwent a gross necropsy. Adrenal glands (dogs only), brain, lungs, pancreas, pituitary gland (dogs only), spleen and thyroid gland (dogs only) of each animal were removed and

weighed. The weights of gonads, heart, kidneys, and liver were also recorded for the 2 mg/L animals and some of the control animals.

The following organs and tissues were taken from all treatment groups. Organs/tissues from the control and 2 mg/L groups were trimmed, sectioned, and stained for histopathological examination. The organs and tissues taken included: adrenal gland (dogs only), aorta, bone, bone marrow, brain, caecum, colon, esophagus, eyes, gonads, heart, kidneys, liver, lung, lymph nodes, skeletal muscle, optic nerves, pancreas, parathyroid, peripheral nerve, pituitary gland (dogs only), prostate gland, salivary glands, small intestines, spinal cord, spleen, sternum, stomach, trachea, thyroid glands (dogs only), urinary bladder, and uterus.

Body weights, body weight changes, and organ weights were analyzed via an analysis of variance with any significant effects further studied with either Scheffe's or Tukey's test of multiple comparison. Organ to body weight ratio and organ to brain weight ratios were studied using the Kruskal-Wallis test with the Kruskal-Wallis multiple comparison.

GLP: No Data

Test Substance: Chlorobenzene, purity not reported

Results: The mean measured analytical concentrations were 0, 0.76, 1.47, and 2.00 mg/L for the 0, 0.75, 1.50, and 2.0 mg/L groups, respectively.

Seven dogs were sacrificed *in extremis*, including two 2 mg/L male, one 1.50 mg/L male, three 2 mg/L females, and one 1.50 mg/L female. One 1.5 mg/L female rat died during the study.

Hypoactivity and conjunctivitis was noted in all 2.0 mg/L and two 1.5 mg/L dogs. One 2 mg/L female dog exhibited glazed eyes.

Hypoactivity was also noted in all test rats. One female rat in the 1.5 mg/L group exhibited diuresis. Two male rats in the 0.75 mg/L group developed skin lesions and alopecia. One male rat in the 2 mg/L group had a red discharge around the nose.

Statistically significant lower mean body weights were observed in the 1.5 mg/L male dogs, 2.0 mg/L female dogs,

1.5 mg/L male rats, and 2.0 mg/L male rats at specific time points during the study. There were no significant final weight change differences between test and control animals.

Mean values for total leukocyte counts for male and female dogs in the 2.0 mg/L groups were lower than the control values after 38 and 88 days of treatment. Mean values for SAP, SGOT, and SGPT from the 2.0 mg/L male and female dogs appeared to be elevated after 38 days of treatment. All other hematology and clinical chemistry endpoints were comparable to control values for rats and dogs. Urinary endpoints were also similar to control values for both rats and dogs.

The 1.5 mg/L female dogs had significantly higher absolute liver weights than controls. The 2.0 mg/L female rats had significantly higher liver to body weight ratios. The 2.0 mg/L male rats had significantly higher absolute kidney weights and the 2.0 mg/L female rats had significantly higher kidney to body weight ratios than control females. The 1.5 mg/L male dogs had significantly lower absolute heart weights. The 2.0 mg/L female rats had significantly higher absolute gonad weights as well as gonad to body weight ratios and gonad to brain weight ratios. The 0.75 and 1.5 mg/L male rats exhibited significantly higher absolute lung weights as well as lower lung to body weight and lung to brain weight ratios. The 1.5 and 2.0 mg/L female dogs had significantly higher absolute pancreas weights. The 0.75 and 1.5 mg/L male rats had significantly lower absolute pancreas weights as well as lower pancreas to body weight and pancreas to brain weight ratios. The 0.75 mg/L female rats had lower absolute pancreas weights and lower pancreas to body weight ratios. The organ weight data for liver, kidneys, heart, and gonads were not available for 14 male and 7 female untreated control rats and all 0.75 and 1.5 mg/L rats.

Except for dogs that were killed while moribund, there were no gross tissue changes in the dogs at necropsy. The dogs killed *in extremis* exhibited yellow discoloration of the aorta, and a few had enlarged and hardened livers.

Slight to moderate vacuolation of hepatocytes were observed in the histology examination in the male and female dogs from the 2.0 mg/L (2/4 males and 3/4 females). The bone marrow of 2 male dogs and 3 female dogs in the 2.0 mg/L

group were aplastic. The bone marrow of another female dog in the 2.0 mg/L group was hypoplastic. Cytoplasmic vacuolation of the epithelium of the renal collecting tubules was observed in the kidneys of 1 male dog and 3 female dogs in the 2.0 mg/L group. Bilateral atrophy of the seminiferous epithelium was observed in the testes of 2 male dogs in the 2.0 mg/L group.

No gross or histological changes attributable to the test substance were observed in the rats.

Reference: Monsanto Co. (1979). Unpublished Industrial Bio-Test Laboratories Report, BLT No. 76-123, "90-Day Subacute Vapor Inhalation Toxicity Study with Monochlorobenzene in Beagle Dogs and Albino Rats" (June 11) (TSCA Fiche OTS0557072).

Reliability: Medium because a suboptimal study design was used.

Supporting Data: Chlorobenzene

Type:	**2-Year Gavage Study**
Species/Strain:	Rats/Fischer 344 Mice/B6C3F1
Sex/Number:	Male and female/50 per sex per group
Exposure Period:	103 weeks
Frequency of Treatment:	5 days/week
Exposure Levels:	0, 30, 60 (male mice) 0, 60, 120 mg/kg (male and female rats, female mice)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

The animals were 6-8 weeks of age at study start. Animals were housed 5/cage. Dose solutions were prepared with corn oil and were prepared every 7-12 days and stored in the dark at 4°C. The stability of the bulk chemical and the concentrations of the dose solutions were confirmed periodically throughout the chronic study. Vehicle control groups (corn oil) were used in the study as well as untreated control groups.

The animals were observed twice daily for mortality and morbundity. Clinical signs of toxicity were recorded monthly. Individual body weights were measured weekly for the first 13 weeks of the study and then monthly thereafter.

At the end of the 104-week exposure period, animals were sacrificed. Tissues removed and examined histologically were: mandibular lymph node, salivary gland, femur, thyroid, parathyroids, small intestine, colon, liver, gall bladder (mouse), prostate, testis, ovary, lungs and bronchi, heart, esophagus, stomach, uterus, brain, thymus, trachea, pancreas, spleen, kidneys, adrenals, urinary bladder, pituitary, and mammary gland. Microscopic examinations occurred in all groups, unless precluded by autolysis or cannibalization.

Probabilities of survival were estimated by the product limit procedure and analyzed for statistically significant test substance related effects by the method of Cox, 1972 (Cox, D. R. (1972). J. R. Stat. Soc., B34:187-220) for comparing 2 groups for equality, and by the method of Tarone, 1975 (Tarone, R. E. (1975). Biometrika, 62:679-682) for assessing dose-related trends. Site-specific tumor frequencies were analyzed between groups for dose-response effects and by pairwise comparisons with controls. Life-table analysis, Peto's incidental tumor test, and Fisher's exact tests and Cochran-Armitage trend tests were used based on the overall proportions of animals bearing tumors at a specific site. A two-sided analysis was used for the survival comparisons and a one-sided analysis was used for the tumor frequency comparisons.

GLP: No Data

Test Substance: Chlorobenzene, purity >99.9%

Results: Mean body weight gains of rats and mice were comparable to the untreated controls and vehicle controls throughout the study. No clinical signs of toxicity were observed.

Survival was decreased in the 120 mg/kg male rats, 30 and 60 mg/kg male mice. The authors state that absence of marked toxic lesions or emaciation in the early-death male animals does not support a causal relationship between chlorobenzene administration and shortened survival. Survival data of the other groups were comparable to the vehicle controls. Deaths of 40 rats and 4 mice were attributed to gavage-related trauma. These deaths were censored from the survival comparisons.

No toxic effects were observed in the male or female mice.

Gavage administration of chlorobenzene appeared to be related to an increased frequency of foreign body aspiration

into the lungs in both sexes of rats. Several of these rats died from gavage-trauma. Inflammatory changes in the lungs appeared to be related to the gavage technique.

Tumors common in aged mice occurred with similar frequency in all groups and were not considered related to chlorobenzene exposure.

The incidence of neoplastic nodules of the liver was significantly increased in the male rats. The increase was significant by dose-related trend tests, and by pairwise comparisons between the vehicle control and the 120 mg/kg group. When the frequency of neoplastic nodules in the male rats was compared to all controls (vehicle and untreated controls combined), the statistical significance of the increase in total liver tumor frequency was strengthened. The frequency of neoplastic nodules in the male rats were 4/50, 2/50, 4/49, and 8/49 in the untreated control, vehicle control, 60 mg/kg, and 120 mg/kg groups, respectively.

Two rare tumor types were also observed in rats receiving chlorobenzene. Transitional-cell papillomas of the urinary bladder were observed in one 60 mg/kg male rat and one 120 mg/kg male rat. Tubular-cell adenocarcinoma of the kidney was observed in one 120 mg/kg female rat. These tumor types were not observed in the controls.

Pituitary tumors occurred at reduced frequency in rats receiving chlorobenzene.

Reference: Kluwe, W. M. et al. (1985). J. Toxicol. Environ. Health, 15:745-767.

Reliability: High because a scientifically defensible or guideline method was used.

5.3 Developmental Toxicity

Developmental Study 1

Species/Strain: Rat/Fischer-344
Sex/Number: Female/30-33
Route of Administration: Inhalation
Exposure Period: Gestation Days 6-15
Frequency of Treatment: 6 hours/day
Exposure Levels: 0, 75, 210, 590 ppm
Method: No specific test guideline was reported; however, a

scientifically defensible approach was used to conduct the study.

After at least a 2-week acclimation period, adult virgin female rats (175-220g) were bred to adult males (1 female to 1 male) of the same strain. Day 0 of gestation was defined as the day sperm was found in a vaginal smear. Rats were housed in wire-bottom cages. Animal rooms were targeted at ~22ºC and relative humidity of ~50%. A 12-hour light/dark photoperiod was employed. Feed and tap water were available *ad libitum* except during inhalation exposures. Treated and control animals were held in separate rooms.

Exposure concentrations were chosen based on the results of a preliminary study in which test atmospheres of 1000 ppm and greater produced severe toxicosis in pregnant rats.

Inhalation exposures were conducted in 4.3 m^3 Rochester-type stainless steel and glass chambers under dynamic airflow conditions (~800 L air/minute). Temperature and humidity of the chambers was targeted at 21ºC and ~50%, respectively. Temperature and humidity were recorded daily for each chamber. Exposure concentrations were generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were then swept into the chamber inlet ducts with compressed air where they were mixed and diluted with incoming air by turbulence. The compressed air supply to the vaporization tubes was preheated to facilitate complete vaporization of the liquid test material. Chlorobenzene concentrations in the chambers were analyzed throughout the exposure period by infrared spectrophotometry. The control group was exposed to filtered room air.

The animals were observed daily for indications of toxicity, and body weights were recorded on gestation days 6, 9, 12, 16, and 21. Food and water consumption were recorded at 3-day intervals beginning on gestation day 6. Test animals were sacrificed on gestation day 21. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses *in utero*, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight, and crown-rump length of each fetus, and any gross external alterations. One-half of each litter was examined for evidence of soft tissue alterations. All fetuses were examined for skeletal

alterations. Maternal liver weights were obtained during the cesarean section.

Statistical evaluation of the frequency of alterations and of resorptions among litters and the fetal population was conducted by the Wilcoxon test as modified by Haseman, J. K. and D. G. Hoel (1974). J. Statist. Comput. Simul., 3:117-135. Statistical analysis of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by either the Dunnett test or the Wilcoxon test, as appropriate.

GLP: No Data

Test Substance: Chlorobenzene, purity 99.982%

Results: Close agreement existed between the mean analytical concentrations and the mean nominal concentrations indicating that test material losses were minimized in the exposure system.

No maternal deaths occurred during the study. No significant effects on general appearance or demeanor were observed during gestation days 6-15. Females in the 590 ppm group gained significantly less weight than controls during gestation days 6-8 (-2±5 grams for the 590 ppm group versus 3±2 grams for the control group). The absolute and relative liver weights on gestation day 21 were significantly increased. No significant effects on body weight gain or liver weights were observed in the 75 or 210 ppm groups.

There was no effect on pregnancy rate. No adverse effects were observed on the mean litter size or incidence of implantations which were undergoing resorption. Actual values for corpora lutea, implantations, number of resorptions, total number of fetuses, total number of live fetuses, mean fetal weight, and sex ratio were not reported.

The incidence of malformations, when considered individually or collectively, was not significantly increased for any concentration group (N=4 in 4 litters in the control, N = 1 in 1 litter at 75 ppm, N = 2 in 2 litters at 210 ppm, N=3 in 3 litters at 590 ppm) With exception of a cleft palate which was observed in a single fetus at 75 ppm, the malformations observed among litters of treated rats were similar to the study control and were at historical incidences for controls. Decreases in the incidence of focal necrosis in

the liver were observed in litters from the 210 and 590 ppm groups. This was not considered to be of toxicological importance. See table below for incidence data.

Skeletal examination of the fetuses revealed increased incidences of some minor variants. The incidence of delayed ossification of centra of the cervical vertebrae was significantly increased over controls in the 75 and 590 ppm groups, but not in the 210 ppm group. Other variants noted included a statistically identified increase in the occurrence of bilobed centra of the thoracic vertebrae and a statistical decrease in the incidence of spurs on the fifth cervical vertebrae in the 590 ppm group. None of the skeletal variants were considered to be indicative of a teratogenic response. See table below for incidence data.

	Concentration (ppm)			
Observation:	0	75	210	590
No. fetuses (No. litters) examined				
External and skeletal exams	241(27)	256(29)	267(27)	258(28)
Soft tissue exams	128(27)	138(29)	141(27)	139(28)
No. fetuses (No. litters) affected				
External alteration:				
Omphalocele*	1(1)	0	0	0
Cleft palate*	0	1(1)	0	0
Soft-tissue alteration:				
Liver, focal necrosis	30(21)	25(18)	22(14)a	19(14)a
Renal agenesis*	1(1)b	0	0	0
Diluted renal pelvis*	0	0	1(1)	2(2)
Right-sided aortic arch*	1(1)b	0	0	0
Microphthalmia *	2(2)b	0	0	1(1)
Anophthalmia*	1(1)	0	1(1)	0
Skeletal alterations:				
Delayed ossification of centra	59(23)	92(27)a	73(23)	103(27)a
Bilobed centra	8(5)	8(7)	3(2)	12(11)a
Cervical spur	25(17)	35(18)	22(14)	13(11)a

* = Considered to be a malformation a = Statistically different from control b = One fetus exhibited renal agenesis, right-sided aortic arch, and microphthalmia.

The maternal NOAEL was 210 ppm. Maternal toxicity was evidenced by decreased body weight gain (590 ppm) and increased absolute and relative liver weights (590 ppm).

Significant increases in 2 skeletal variants (delayed ossification of vertebrae centra and bilobed thoracic centra) were indicative of a slight delay in skeletal development among the fetuses of the dams exposed to 590 ppm (a maternally toxic dose). The fetal NOAEL was 210 ppm. Skeletal variations, indicative of mild fetotoxicity, were observed at doses that also resulted in mild maternal toxicity (590 ppm).

Chlorobenzene was not a unique developmental toxin.

Reference: John, J. A. et al. (1984). Toxicol. Appl. Pharmacol., 76:365-373.

Reliability: High because a scientifically defensible or guideline method was used.

Developmental Study 2

Species/Strain: Rabbit/New Zealand White

Sex/Number: Female/30

Route of Administration: Inhalation

Exposure Period: Gestation Days 6-18

Exposure Levels: 0, 75, 210, 590 ppm (first study); 0, 10, 30, 75, 590 ppm (second study)

Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

After at least a 2-week acclimation period, female rabbits (3.5-4.5 kg) were artificially inseminated. Day 0 of gestation was defined as the day of artificial insemination. Rabbits were housed in wire-bottom cages. Animal rooms were targeted at ~22ºC and relative humidity of ~50%. A 12-hour light/dark photoperiod was employed. Feed and tap water were available *ad libitum* except during inhalation exposures. Treated and control animals were held in separate rooms.

Exposure concentrations were chosen based on the results of a preliminary study in which test atmospheres of 1000 ppm

and greater produced severe toxicosis in pregnant rats.

Inhalation exposures were conducted in 4.3 m^3 Rochester-type stainless steel and glass chambers under dynamic airflow conditions (~800 L air/minute). Temperature and humidity of the chambers was targeted at 21ºC and ~50%, respectively. Temperature and humidity were recorded daily for each chamber. Exposure concentrations were generated by metering the liquid test material at controlled rates into vaporization tubes. Vapors from the tubes were then swept into the chamber inlet ducts with compressed air where they mixed and diluted with incoming air by turbulence. The compressed air supply to the vaporization tubes was preheated to facilitate complete vaporization of the liquid test material. Chlorobenzene concentrations in the chambers were analyzed throughout the exposure period by infrared spectrophotometry. The control group was exposed to filtered room air.

The animals were observed daily for indications of toxicity and body weights were recorded on gestation days 6, 9, 12, 15, 19, and 29. Test animals were sacrificed on gestation day 29. The uterine horns were exteriorized and the following data were recorded: number and position of fetuses *in utero*, number of live and dead fetuses, number and position of resorption sites, number of corpora lutea, sex, body weight, and crown-rump length of each fetus, and any gross external alterations. One-half of each litter was examined for evidence of soft tissue alterations. All fetuses were examined for skeletal alterations. Maternal liver weights were obtained during the cesarean section.

Statistical evaluation of the frequency of alterations and of resorptions among litters and the fetal population was conducted by the Wilcoxon test as modified by Haseman, J. K. and D. G. Hoel (1974). J. Statist. Comput. Simul., 3:117-135. Statistical analysis of the percentage of pregnancy and other incidence data were conducted by the Fisher exact probability test. Analyses of other data were made by parametric or nonparametric analysis of variance followed by either the Dunnett test or the Wilcoxon test, as appropriate.

Due to the presence of a variety of external and visceral malformations among only the exposed groups, a second study was initiated to further evaluate the test material. The second study was conducted at lower dose levels in the same

manner as described above.

GLP: No Data

Test Substance: Chlorobenzene, purity 99.982%

Results: Close agreement existed between the mean analytical concentrations and the mean nominal concentrations indicating that test material losses were minimized in the exposure system.

Study 1: In the first study, there were increased absolute and relative liver weights in rabbits exposed to 210 or 590 ppm. There was no effect of treatment on pregnancy rate, mean litter size, or the incidence of resorptions. Pregnancy rates for the 0, 75, 210, and 590 ppm groups were 97% (29/30), 93% (28/30), 93% (28/30), and 97% (29/30), respectively. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

	Concentration (ppm)			
Observation	0	75	210	590
Corpora lutea:	NR	NR	NR	NR
Implantation sites/dam:	9±2	9±2	8±2	9±2
% litters with resorptions	41 (11/27)	58 (15/26)	41 (11/27)	41 (12/29)
Fetuses/litter	8±2	8±2	7±2	8±2
Total No. of Live Fetuses:	NR	NR	NR	NR
Mean Fetal Weight (g):	35.67	35.51	38.17	37.58
Sex Ratio:	NR	NR	NR	NR
NR = Not Reported				

Exposure to 75, 210, or 590 ppm resulted in a variety of malformations in all groups at incidences slightly higher than historical controls. The incidence of malformation at these concentrations was 11 (in 6 litters), 8 (in 7 litters), 6 (in 5

litters), and 8 (in 7 litters). Forelimb flexure, the malformation most often observed, occurred more often among controls than among any of the treatment groups, as did malformations of the skeletal system. The latter included hemivertebrae, missing or fused vertebrae, crooked long bones, and a variety of rib malformations. However, there were several cases of external and visceral malformations which were scattered among the exposed groups. A single case of spina bifida and a low incidence of heart anomalies were observed in the 210 and 590 ppm groups, whereas these malformations were not observed among the control group. The fetus at 210 ppm exhibited a ventricular septal defect, one fetus at 590 ppm also showed a septal defect whereas a second fetus exhibited a persistent truncus arteriosus. In several cases, affected fetuses had more than one malformation. There was no apparent trend for a dose-related increase in the occurrence of any single malformation, with the possible exception of the heart anomalies previously mentioned. The incidence of skeletal alterations were unaffected by exposure, with the exception of an increased incidence of extra ribs in the high dose animals (113 in 26 litters vs. 79 in 24 control litters). This alteration was considered to be a variant in this species. To determine if findings were true effects of treatment, the study was repeated.

Study 2: To ascertain whether the low incidence of head and facial anomalies and heart defects was a true effect of treatment, additional groups were exposed to 0, 10, 30, 75, or 590 ppm. An increase in liver weight of the maternal animals was observed, as was the case in the first study. Pregnancy rates for the 0, 10, 30, 75, and 590 ppm groups were 94% (30/32), 93% (27/29), 97% (29/30), 93% (28/30), and 97% (31/32), respectively. A significant increase in the percentage of implantations undergoing resorption was observed in the 590 ppm group (61% vs 41%). Since the 61% value was within the historical control range for the laboratory (mean 40%, range 19-67%), the apparent increase was not interpreted to be indicative of an embryotoxic effect. The resorption rate was not significantly altered at the lower exposure concentrations and was unaffected in the first study. Fetal body measurements were not adversely affected by treatment. A summary of reproductive outcomes (means/litter unless otherwise noted) is provided in the table below:

	Concentration (ppm)				
Observation	0	10	30	75	590
Corpora lutea:	NR	NR	NR	NR	NR
Implantation sites/dam:	8±3	8±3	9±3	8±2	8±2
% litters with resorptions	41 (11/27)	48 (12/25)	50 (13/26)	50 (14/28)	61 (19/31)
Fetuses/litter	8±3	7±3	7±3	8±3	7±2
Total No. of Live Fetuses:	NR	NR	NR	NR	NR
Mean Fetal Weight (g):	37.44	38.87	37.30	38.49	39.29
Sex Ratio:	NR	NR	NR	NR	NR
NR = Not Reported					

The incidence of malformations from the exposed groups, when considered individually or collectively, was not significantly increased compared to the control group. The incidence of malformations in fetuses from animals treated with 0, 10, 30, 75, or 590 ppm was 14 (in 11 litters), 3 (in 3 litters), 14 (in 8 litters), 7 (in 5 litters), and 14 (in 5 litters). Fetuses with external, soft tissue, and skeletal malformations were observed among all groups including controls. There was no trend for clustering of any anomalies among the exposed groups in the second study. There were 7 fetuses with ablepharia (missing eye lid) in the 590 ppm group; however, this was a single litter effect. This anomaly was not observed in the first study. Heart anomalies were observed in controls as well as some exposed groups and there was no indication of a dose-related effect in the occurrence of heart anomalies (2 in controls and 0-2 in treated). No head or facial abnormalities were observed in any group.

Skeletal examination revealed a significant increase in the

incidence of extra ribs in the 10 ppm group. This alteration is considered a skeletal variant and the increased incidence in the lowest exposure group was not considered to be toxicologically important.

The maternal NOAEL was 75 ppm. Maternal toxicity was evidenced by significant increases in liver weight at 210 and 590 ppm. The fetal NOAEL was > 590 ppm. Therefore, the test material was not teratogenic.

Reference: John, J. A. et al. (1984). Toxicol. Appl. Pharmacol., 76:365-373.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for Developmental Toxicity: None Found.

5.4 Reproductive Toxicity:

Study Type:	**28-Day Inhalation Study**
Species/Strain:	Rats/Sprague-Dawley CD
Sex/Number:	Male and female/5 per sex per group
Route of Administration:	Inhalation
Exposure Period:	28 consecutive days
Frequency of Treatment:	6 hours/day
Exposure Levels:	0, 0.4, 1.5, 6.0 mg/L (0, 94, 381, 1585 ppm)
Method:	A 28-day inhalation study was conducted according to the following guidelines: Method B8, Annex V of the EEC Commission Directive 84/449/EEC and OECD Guideline 412. See Section 5.2 for details on study design. At study termination, rats underwent both gross and microscopic examinations. Reproductive organs included in the histopathological evaluation included testes with epididymis, prostate, seminal vesicles, mammary gland, ovaries, and uterus. The testis with epididymides and ovaries were weighed.
GLP:	Yes
Test Substance:	Fluorobenzene, purity not reported
Results:	No compound-related effects on the reproductive organs of either male or female rats were observed.
Reference:	Safepharm Labs. Ltd. (1993). Project No. 121/194, "28-Day Subacute (nose-only) Inhalation Toxicity Study in the Rat" (January 12) (cited in TSCA Fiche OTS0572061).

Reliability: High because a scientifically defensible or guideline method was used.

Supporting Data: Chlorobenzene

Study Type: 2-Generation Reproduction Study
Species/Strain: Rats/Sprague-Dawley CD
Sex/Number: Male and female/30 per sex per group
Route of Administration: Inhalation
Exposure Period: F0 and F1 generation: during mating period, gestation days 0-20, and lactation days 4-21
Frequency of Treatment: 6 hours/day, 7 days/week
Exposure Levels: 0, 50, 150, 450 ppm (0, 234, 702, 2105 mg/m^3)
Method: No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study.

Rats were 6 weeks of age on the first day of exposure.

Animal cages were placed into 6 m^3 stainless steel and glass inhalation chambers during exposure. The chambers were operated dynamically at an air flow rate of at least 2140 L/min. Atmospheres were generated by feeding the test material into an atomizing nozzle via a fluid metering pump. The vaporized test material was diluted with preconditioned air prior to entering the exposure chamber. Exposure concentrations were periodically measured via an organic vapor analyzer.

Animals were exposed for 10 weeks prior to mating. For mating, 1 male was cohoused with 1 female within the same treatment group at night until evidence of mating was observed or until 10 nights had elapsed. If mating hadn't occurred, the female was housed with another male within the same treatment group for another 10-day interval. The presence of a copulatory plug and/or presence of sperm in the vaginal smear were considered evidence of successful mating. Exposure of males and females continued through gestation and lactation except from gestation day 20 through lactation day 4.

After F1 pups were weaned, the F0 animals were sacrificed. Some F1 weanlings were selected to be used as parents for the next generation. These pups started treatment (at the same dose level as their parents) 1 week after weaning and

treatment was continued for at least 11 weeks prior to mating. The rest of the treatment regimen was the same as for the F0 generation. F1 adults were killed after weaning of the F2 pups. All F2 pups were killed at weaning and necropsied.

All adult and weanling animals were observed for mortality and clinical signs twice daily. Detailed clinical signs and male body weights were recorded weekly. Female body weights were recorded periodically throughout the study. Food consumption was measured weekly throughout the growth period.

Litters were examined twice daily for general appearance of the pups and for dead pups. The number of pups per litter and pup sex distribution was periodically recorded. Litters were culled to 8 on day 4 of lactation. Individual pup weights were periodically recorded throughout lactation.

Complete gross examinations were conducted on all F0 and F1 parents, all F1 weanlings not selected to become adults, and all F2 weanlings. Liver and brain weights of the F0 and F1 adults were recorded. Liver, kidneys, pituitary gland, and reproductive organs (males – testes, epididymides, seminal vesicle, prostate; females – vagina, uterus, ovaries) were examined microscopically for all F0 and F1 adults in the 0 and 450 ppm groups. Liver, kidneys, and testes of male rats in the 50 and 150 ppm groups were also examined histologically.

The mating index for males and females, pregnancy rate, and fertility index for males were calculated for each of the 2 matings. Pup survival indices at various intervals during lactation were also calculated.

Mean body weights, food consumption, organ weights, organ to body weight ratios, gestation lengths, and numbers of offspring were evaluated with the following methods: Bartlett's test followed by either one-way analysis for variance and Dunnett's test or the Kruskal-Wallis test and the Dunn's summed rank test and/or Jonckheere's test. Pup viability indices and pup survival indices were transformed using the arc sine transformation. Incidence data, which included pregnancy rates, fertility indices, mating indices, and litter survival indices, were analyzed using contingency tables. A standard chi-square analysis was performed and

then treatment groups were compared to the control group with the Fisher exact test. An Armitage test for linear trend was also performed.

GLP: No Data

Test Substance: Chlorobenzene, purity 99.9%

Results: Cumulative mean analytical concentrations were 51, 151, and 451 ppm for the 50, 150, and 450 ppm groups, respectively.

No mortality, no substance-related changes of body weights or food consumption were observed. Mating and fertility indices appeared unaffected by treatment. In the F1 litter, pup and litter survival indices were comparable to control. In the F2 litters, a slight decrease in pup survival index was observed in the high concentration group only. This was attributed to 2 dams; 1 dam lost 12 of 15 pups during lactation, and the other dam lost all 10 pups during lactation. The authors assert that if this had been a compound-related effect, more litters would have been affected. Therefore, the pup survival indices for control and treated animals were considered comparable.

Significant increases in mean absolute or relative liver weight were observed in the F0 and F1 generations in the 150 and 450 ppm groups. The mean relative liver weight for males in the 50 ppm group (F1 generation) was also significantly elevated. Histopathological examination of the liver revealed hepatocellular hypertrophy in the centrilobular region in the 150 and 450 ppm male groups only. The authors state that hepatocellular hypertrophy may be a reflection of the ability of chlorobenzene to induce hepatic enzymes. In the absence of microscopic changes, the biological significance of the changes in the liver weight of the 50 ppm males and the 150 and 450 ppm females was unclear.

An increase in the incidence of small flaccid testes and dilated renal pelvis was observed in the 450 ppm group for both the F0 and F1 adults. For small flaccid testes the incidences observed were: F0 – 0, 1, and 3 for the 50, 150, and 450 ppm groups and F1 – 0, 1, and 5 for the 50, 150 and 450 ppm groups, respectively. The incidence of dilated renal pelvis appeared to be elevated in the F0 450 ppm males (1, 1, 2, 5 for the 0, 50, 150, and 450 ppm groups, respectively). The incidence in F0 females was 5, 4, 6, and 5 for the 0, 50, 150, and 450 ppm groups, respectively. In the F1 adults, the

increase in incidence was not dose-related (F1 males: 1, 4, 6, 4 for the 0, 50, 150, and 450 ppm; F1 females: 0, 1, 2, 2 for the 0, 50, 150, and 450 ppm, respectively). Since the relationship in the F1 generation was not dose-related, the observation was considered an equivocal effect.

Hepatocellular hypertrophy and renal changes were observed among F0 and F1 males exposed to 150 and 450 ppm. The incidence of hepatocellular hypertrophy for F0 adults was 0, 0, 5, and 14 for the 0, 50, 150, and 450 ppm groups, respectively. The incidence of hepatocellular hypertrophy for F1 adults was 2, 0, 3, and 7 for the 0, 50, 150, and 450 ppm groups, respectively. All affected male rats showed only minimal to mild hepatocellular hypertrophy. Only 1 450 ppm female in the F0 generation had hepatocellular hypertrophy. Renal degeneration and inflammatory lesions were limited to male rats.

The incidence of bilateral degeneration of the testicular germinal epithelium was increased among F0 males in the 450 ppm group and this lesion was observed only unilaterally in the 150 and 450 ppm group F1 adults. The authors state that the relationship of these testicular changes to chlorobenzene exposure was unclear because there did not appear to be any increase in intensity and/or incidence of testicular lesions among F1 adults that had longer exposure.

No adverse effects on reproductive performance or fertility were observed in male or female rats.

Parental NOEL: 50 ppm
F1 Offspring NOEL: 50 ppm
F2 Offspring NOEL: > 450 ppm

Reference: Nair, R. S. et al. (1987). Fundam. Appl. Toxicol., 9:678-686.
Reliability: High because a scientifically defensible or guideline method was used.

5.5 Genetic Toxicity

Type: ***In vitro*** **Bacterial Reverse Mutation Assay**
Tester Strain: *Salmonella typhimurium* strains TA98, TA100, TA1535
Exogenous Metabolic Activation: With and without Aroclor®-induced rat and hamster liver S9
Exposure Concentrations: 0, 5, 10, 25, 33, 50, 100, 250, 333, 500, 750, 1000, 1666 µg/plate (TA100)

	0, 5, 10, 25, 33, 50, 100, 333, 500, 1000, 1666 µg/plate (TA98) 0, 5, 10, 50, 100, 250, 500, 750 µg/plate (TA1535)
Method:	No specific test guideline was reported; however, a scientifically defensible approach was used to conduct the study. A preincubation modification of the *Salmonella*/mammalian microsome mutagenicity (Ames test) was conducted. The tests were conducted in 2 laboratories under contract to the NTP. The protocol used and the data evaluation criteria have been previously described (Zeiger, E. et al. (1992). Environ. Mol. Mutagen., 16(Suppl. 18):1-14). Chemicals were tested in a preincubation procedure in strains TA98 and TA100 without metabolic activation and with activation provided by Aroclor-induced rat and hamster liver homogenates (S9). If a positive response was seen in 1 of these 2 strains, the strain/metabolic activation combination producing that response was repeated, and no further testing was performed. If no positive responses were seen, the chemical was tested in strain TA1535.
GLP:	Unknown
Test Substance:	Fluorobenzene, purity not reported
Results:	Positive
Remarks:	A positive result with hamster liver activation was observed in strains TA100 and TA1535.
Reference:	Zeiger, E. and B. H. Margolin (2000). Regul. Toxicol. Pharmacol., 32:219-225. NTP (1992). Annual Report for Fiscal Year.
Reliability:	High because a scientifically defensible or guideline method was used.
Type:	***In vitro* Bacterial Reverse Mutation Assay**
Tester Strain:	*Salmonella typhimurium* strains TA98, TA1538, TA1537, TA100, TA1535
Exogenous Metabolic Activation:	With and without rat liver S9
Exposure Concentrations:	0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24 µL
Method:	No specific test guideline was reported; however, a

scientifically defensible approach was used to conduct the study. A pour plate method for quantitative determination following the Ames procedure was conducted.

Each concentration of test substance was added to sterile test tubes containing 3-6 x 10^7 bacterial cells and S9 mix or sodium phosphate buffer (pH 7.4). The mixture was preincubated in a shaker water bath at 37ºC for 15 minutes, then added to molten top agar (45ºC). The tubes were mixed well and then the contents were immediately poured onto the surface of a minimal agar plate. Plates were inverted and incubated at 37ºC in the dark for 3 days. Colonies of his+ revertants were counted after incubation. If the chemical induced more than twice the number of revertant colonies compared to the control plate, it was considered mutagenic.

All tests were performed in duplicate and repeated at least 3 times separately.

The test compound was dissolved in dimethyl sulphoxide (DMSO) to obtain appropriate test concentrations. The control plate contained DMSO. The S9 mix contained S9, $MgCl_2$, KCl, glucose 6-posphate, NADH, NADPH, and sodium phosphate.

N-Ethyl-N'-nitro-N-nitrosoguanidine, 2-nitrofluorene, 9-aminoacridine, and 2-aminoanthracene were used as positive controls.

GLP: Unknown
Test Substance: Fluorobenzene, purity 99%
Results: Negative
Remarks: No additional data.
Reference: Shimizu, M. et al. (1983). Mutat. Res., 116(3-4):217-238.
Reliability: High because a scientifically defensible or guideline method was used.

Additional References for *In vitro* Genetic Toxicity: None Found.

Type: ***In vivo*** **Mouse Micronucleus Assay**
Species/Strain: Mice/NMRI
Sex/Number: Male and female/42 per sex
Route of Administration: Oral
Concentrations: Initial experiment determined the maximum dose of 5000 mg/kg.
Method: The procedures used in the test were based on the

recommendations of the following guidelines: OECD Guideline 474, EEC Directive 84/449, and EPA 40 CFR Subpart F.

Mice weighed approximately 30 g. The test material was suspended in corn oil, which also served as the vehicle control. Approximately 18 hours before treatment, the animals received no food, but continued to receive water *ad libitum*. A single oral administration of 5000 mg/kg was given. After 24, 48, and 72 hours, the bone marrow smear was prepared. Animals were sacrificed and the femora were removed. The epiphyses were cut off and the marrow was flushed out with fetal calf serum. The cell suspension was centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded. A small drop of the resuspened cell pellet was spread on a slide. The smear was air dried and then stained with May-Grünwald/Giemsa. Cover slips were mounted and at least 1 slide was made from each bone marrow sample.

Although 6 mice per gender and time were administered the test substance, only 5 animals per gender and time were evaluated. 1000 micronuclei polychromatic erythrocytes (PCE) were evaluated using microscopes with 100x oil immersion objectives. The remaining animal of each test group was evaluated only if an animal that that time point had died during the test.

The ratio between polychromatic and normochromatic erythrocytes (NCE) were determined and reported as the number of NCE per 1000 PCE.

Cyclophosphamide (CPA) was used as a positive control in the study.

The test material was classified as mutagenic if it induced a significant increase in the number of micronucleated PCEs in at least 1 of the test points. A test material producing no statistically significant increase in the number of micronucleated PCEs at any of the test points was considered non-mutagenic. Statistical analysis was conducted with the use of the Mann-Whitney test.

The 5000 mg/kg dose level was chosen based on results from a rangefinding experiment which determined that 5000 mg/kg was close to the maximum tolerated dose. The

	number of NCEs per 1000 PCEs was increased compared to the control indicating a cytotoxic effect and 1 of the treated males died during the rangefinding test.
GLP:	Yes
Test Substance:	Fluorobenzene, purity 99.7%
Results:	Negative
Remarks:	In comparison with controls, there was no significant increase in the frequency of detected micronuclei at any preparation interval.
Reference:	BG Chemie (1991). Cytotest Cell Research Gmbh & Co. Report No. 126, "Micronucleus Assay in Bone Marrow Cells of the Mouse" (July 1).
Reliability:	High because a scientifically defensible or guideline method was used.

Additional Reference for *In vivo* Genetic Toxicity:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

BG Chemie (1993). Toxikol. Bewertung, No. 126 (August) (cited in Bayer, E. and G. Fleischhauer (1993). Chemosphere, 26(10):1789-1822).